University of London
London School of Hygiene & Tropical Medicine
Infectious Disease Epidemiology Unit

THE EPIDEMIOLOGY OF HERPES ZOSTER IN AN URBAN POPULATION

Sara Loraine Monro Thomas

Thesis submitted for the degree of
Doctor of Philosophy

February 2003

Abstract

A community-based case-control study was carried out in South London, to investigate the determinants of herpes zoster in adults without underlying immunosuppression. Incident zoster cases were identified from general practices. Two controls were selected per case, matched by age, sex, and practice. Participants were interviewed to determine exogenous contacts with varicella and with children as proxies for varicella contacts; ethnicity, country of birth and age at varicella; micronutrient intake and intake of fruit and vegetables; ultraviolet radiation (UVR) exposure in childhood and in the last year, and stressful events. Odds ratios were estimated using conditional logistic regression.

Data from 244 cases and 485 controls were analysed. On multivariable analysis, contacts with children significantly protected against zoster - most heavily exposed individuals were at one fifth the risk of unexposed individuals, and this appeared to be mediated by increased exposure to varicella cases. Childhood UVR exposure was associated with a strongly increased risk of zoster. Individuals eating fresh fruit less than once a week were at a six-fold risk of zoster compared to those with highest intakes, and individuals aged >60 years with a high combined micronutrient intake were protected against zoster. Individuals experiencing an incident stressful event in the last two months were at more than twice the risk of zoster, and stressful events in the last year were associated with increased risk amongst elderly individuals. The protective effect of Afro-Caribbean ethnicity was mostly explained by increased child contacts and high fresh fruit intake.

This study identified new risk factors for zoster. Those factors that were restricted to older individuals may also be determinants of immunosenescence. The findings suggest that widespread varicella vaccination of children could lead to increased incidence of adult zoster by decreasing exogenous varicella exposures. Other implications for future research and for public health policy are discussed.

Contents

List of Tables

Tables in the text

Tables for analyses reported in Chapter 6

List of Figures

List of abbreviations

95% CI	95% Confidence intervals
BMI	Body mass index
DNA	Deoxyribonucleic acid
DTH	Delayed type hypersensitivity
EV	Early varicella
FFQ	Food frequency questionnaire
GP	General practitioner
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
IDA	Integrated Dietary Analysis (software)
IL	Interleukin
LRT	Likelihood ratio test
LV	Late varicella
M & W	McCance & Widdowson's (Composition of Foods Tables)
MED	Minimal erythemal dose
MSGP	Morbidity Statistics in General Practice
NDNS	National Diet and Nutrition Surveys
NK	Natural killer (cells)
OR	Odds ratio
PCR	Polymerase chain reaction
PI	Principal investigator
PHN	Post-herpetic neuralgia
RCGP	Royal College of General Practitioners
TH_1	T-helper cell type 1 subset
TH_2	T-helper cell type 2 subset
TNF	Tumour necrosis factor
UK	United Kingdom
US	United States (of America)
UVA	Ultraviolet A radiation
UVB	Ultraviolet B radiation
UVR	Ultraviolet radiation
VZV	Varicella zoster virus

Acknowledgements

I would like to thank my supervisor, Andy Hall, for teaching me an enormous amount about epidemiology, for inspiring me to do this thesis, and for providing invaluable technical guidance and constructive comments. Jerry Wheeler was a calm and enabling statistical advisor, who helped me find a way through the mass of data. I would also like to thank Oona Campbell for providing emotional support and reassurance, for her discerning feedback on drafts, and for giving much needed practical advice on how to complete the thesis.

Dr Judy Breuer of the London Hospital kindly agreed to undertake the PCR analyses for the study, and gave helpful suggestions on the diagnosis of zoster. Dr Mike Nelson of Kings College (London) came up with the solution for transforming the dietary data, and then generously gave of his time to teach me how to use the IDA software.

The Medical Research Council granted me a studentship that enabled me to undertake this work. Professor Michiaki Takahashi of the Research Foundation for Microbial Diseases (Osaka University) generously stepped in with financial assistance for the fourth year of the study, for which I am very grateful.

This research would not have been possible without the individuals who gave up their time to participate in the study, and the staff of the 24 participating general practices: the Albion Street Health Centre; Aylesbury Partnership (all three sites); Belmont Hill Surgery; Brixton Hill Group Practice; Camberwell Green Surgery; Crown Dale Medical Centre; Grange Road Practice; Downham Way Surgery; Forest Hill Group Practice; Isidore Crown Health Centre; Jenner Health Centre; Lee Road Surgery; Mawbey Brough Health Centre; New Surgery, Clapham; Paxton Green Health Centre; Queens Road Partnership (both sites); Sternhall Lane Surgery; Sydenham Green Group Practice; and the Torridon Road Surgery.

Finally, I would like to acknowledge the unstinting support I received from Anthony, who for six years coped with my absences and interminable conversations about zoster. He listened, cooked wonderful food, and provided much needed humour that enabled me to see this project through to completion.

1: INTRODUCTION

Herpes zoster (shingles) occurs as a result of reactivation of varicella zoster virus (VZV) which has remained dormant since primary infection associated with varicella (chickenpox). Zoster occurs frequently in ageing populations, with an estimated lifetime risk of 23-30% amongst individuals living in the United Kingdom.[1,2] It causes significant acute morbidity, including dermatomal pain, vesicular rash and altered sensation. The commonest sequela is postherpetic neuralgia (pain lasting longer than one month after rash onset), which may follow zoster in approximately 50% of individuals aged over 60 years, and which can persist for years.[3,4] It has been estimated that zoster results in a total of 19,966 quality-adjusted life years lost annually in England and Wales, and costs health care providers more than £47.6 million per year.[5]

Oral antiviral therapies can be given to individuals with zoster in an attempt to limit acute pain and reduce the risk of post-herpetic neuralgia. There is evidence that antivirals have some effect if given within 72 hours of rash onset.[6-8] However, many patients present too late to benefit from early therapy, and the frequent occurrence of prodromal pain in zoster indicates that neuronal damage often occurs before rash develops. Given the common occurrence of PHN and difficulties in its treatment, strategies to reduce the incidence of zoster are needed. This would be facilitated if there were a good understanding of the determinants of VZV reactivation.

Latent VZV virus is thought to reactivate as a result of declining specific cell-mediated immunity.[9-12] It follows that individuals with diminished cell-mediated immunity due to immunosuppressive conditions or therapies are at higher risk of zoster.[13-15] However, these individuals only constitute 1-11% of zoster cases in population-based studies,[4,16-22] and the determinants of VZV reactivation in people without underlying immunosuppression are largely unknown. One of the few established risk factors is age - the risk of zoster increases markedly in elderly individuals.[4,23] This may be due to the generalised loss of cell-mediated immunity which occurs as part of the ageing process, or to waning of VZV-specific immunity over time. Little is known about the determinants of either generalised or VZV-specific immune decay.

Research is therefore needed to elucidate risk factors for VZV reactivation as zoster. As well as informing strategies to prevent zoster, this research could provide information on two additional issues:

1. A vaccine is available against varicella, and its introduction has been considered by many countries.[24] A critical issue for any proposed varicella vaccination programme is whether continued exposure to cases of varicella protects individuals with latent infection from zoster, by exogenous boosting of VZV-specific immunity. If this were the case, widespread introduction of varicella vaccination could lead to an increase in the incidence of zoster. Research findings on the effect of exposure to varicella on the risk of zoster would allow modification of existing mathematical models which represent the transmission of varicella in an age structured population, including equations describing the incidence of herpes zoster.[25,26] These models can be used to explore the potential impact of introducing varicella vaccination in the UK and elsewhere, and to inform estimates of the cost effectiveness of vaccination.[5]

2. The public health importance of diseases of the elderly is increasing in the United Kingdom as the population ages. Studies are needed to investigate extrinsic factors that contribute to the increasing incidence of disease with age, including increased susceptibility to infections. The generalised loss of cell-mediated immunity with age underlies this increasing morbidity. If reactivation of VZV as zoster in older individuals is a marker of generalised immune senescence, investigation of risk factors for zoster may also provide insight into the determinants of the generalised decline in immunity in the elderly.

This thesis is a report of an investigation into the determinants of zoster in individuals with no known underlying immunosuppression. In Chapter 2, the literature on zoster is summarised – this includes the biology and natural history of VZV infection, and existing descriptive and analytical epidemiological studies of zoster. The rest of the thesis describes a community-based matched case-control study of risk factors for zoster that was carried out in an urban United Kingdom population. Cases were adults with recently diagnosed zoster presenting to general practices in 1997-98. For each case, two controls (with no history of zoster) were selected, individually matched by age, sex, and practice. Participants were interviewed at home, using a standardised questionnaire. Information was sought on 1) factors that might protect against zoster by boosting specific immunity, 2) possible determinants of generalised loss of cell-mediated immunity and 3) other factors that might affect risk of zoster. Chapter 3 provides details of the methods used, including the study objectives, the study setting, recruitment of cases and controls, data collection and management, and the overall analytical strategy. Two groups of data required complex data transformation, and this is discussed in Chapter 4. The descriptive results are presented in Chapter 5. Chapter 6 contains the analyses of risk factors for zoster, and is divided into sections – the first five of

these describe analyses of the five main subgroups of data, each with details of the specific hypotheses tested, analytical strategies used, results of univariable and multivariable analyses, and discussion of the findings. The final section reports the findings from a combined model, which contained selected variables from the five sub-models. In Chapter 7, the main findings are summarised, the strengths and potential weaknesses of the study are analysed, and the implications for future research and public health practice are discussed.

2. LITERATURE REVIEW

2.1 BIOLOGY AND NATURAL HISTORY OF HERPES ZOSTER

Varicella zoster virus (VZV) is an alpha-herpes virus with a double-stranded DNA genome of approximately 125,000 base pairs.[27] Primary infection is manifested as varicella (chickenpox), following which the virus establishes latency in dorsal root ganglia, residing predominantly in neurones.[28-30] Reactivation of latent virus, often many decades after primary infection, results in herpes zoster (shingles). Control of the virus in the latent form is thought to be maintained by specific cell-mediated immunity. Evidence for this includes: 1) increasing risk of zoster and declining VZV-specific cell-mediated responses (reduced lymphoproliferation and delayed type hypersensitivity reactions) both occur with age;[4,9,10,23] 2) immunocompetent individuals at the onset of zoster have low VZV-specific cell-mediated responses compared to non-zoster controls,[11,12] and 3) individuals with diminished cell-mediated immunity due to immunosuppressive conditions experience zoster more frequently compared to immunocompetent individuals.[13-15] In contrast to the low cell-mediated responses, high levels of VZV-specific antibody are present at onset of zoster.[31,32]

Reactivation in a single ganglion is thought to result in replication of the virus, which travels down sensory neural axons to the skin supplied by the dorsal root.[33] The resulting erythematous rash of zoster therefore has a dermatomal unilateral distribution. The rash is typically limited to a single dermatome, but eruptions in the adjacent or in a separate dermatome have been described.[34] The extent of rash within the dermatome varies; a large proportion of the dermatome is often involved but less extensive rash can occur, and very rarely there may be no rash visible (zoster sine herpete).[35-37] The rash is initially maculopapular before developing into characteristic vesicles followed after about a week by crusts, although occasionally the papules persist without vesicle formation.[38] The rash is usually accompanied (and often preceded) by dermatomal pain and/or paresthesia.[35]

Specific cell-mediated immunity increases after zoster.[12] Second attacks of zoster are uncommon in the immunocompetent, but were reported in 0.8-5% of individuals in population-based studies with follow-up of 9-30 years.[4,21,34] Hope-Simpson extrapolated from his own data to estimate that amongst one thousand individuals who lived to the age of 85 years, approximately half would experience a single episode of zoster, ten individuals would experience a second episode, and one individual would experience a third episode.[34] However, recurrent zoster occurs more commonly in the immunosuppressed.[39]

The major sequela of zoster is chronic pain (post herpetic neuralgia, or PHN), which is commonly defined as pain lasting at least one month after rash onset.[35] This occurs increasingly with age and may be experienced by approximately 50% of individuals over the age of 60 years.[4,35] The mechanism for PHN is unclear, but is thought to involve virus-induced inflammation and death of neurones.[40] Uncommon neurological complications of zoster include contralateral hemiparesis, myelitis, motor neuropathies, cranial nerve palsies and encephalitis.[37] Zoster of the ophthalmic branch of the trigeminal nerve can result in corneal scarring or secondary panophthalmitis.[4,41] In immunosuppressed individuals, widely disseminated rash with visceral involvement may occur.[42]

Once established, PHN can persist for years.[4] Oral antiviral therapies are often given during the acute phase of zoster in an attempt to reduce the incidence of PHN. Randomised controlled trials have demonstrated that prompt administration of antivirals may reduce acute pain, limit the extent of rash and hasten rash healing, but may only have limited efficacy against PHN.[6-8] Prodromal pain is often reported in zoster, and this suggests that neuronal damage occurs early in many people, even before they develop rash. In addition, patients often present more than 72 hours after rash onset, at which time oral antivirals are less effective. Therefore, zoster-associated morbidity may be better avoided by trying to prevent reactivation of VZV, rather than by treating manifestations of reactivated infection.

2.2 DIAGNOSIS OF HERPES ZOSTER

The differential diagnosis of clinical zoster includes herpes simplex virus (HSV) infection, impetigo, and contact dermatitis. The major consideration in adults is to exclude HSV infection. The characteristic dermatomal distribution and accompanying pain usually identifies individuals with zoster. Other clinical differences between zoster and HSV infection include:[4,34,43]

- *Frequency of recurrence:* second episodes of zoster are uncommon, whereas HSV recurrences are frequent, usually occurring in the same site;
- *Site of rash:* zoster commonly occurs within the thoracic or lumbar dermatomes or in the distribution of the ophthalmic branch of the trigeminal nerve, whereas HSV infection most frequently arises as a small patch of rash on the buttocks or perioral area;
- *Pain:* zoster is usually accompanied by acute pain, and often results in PHN. Simplex infection is typically accompanied by a sensation of burning, tingling or itching, and does not lead to PHN.

In a few cases it may not be possible to distinguish zoster from HSV infection by clinical criteria alone. This is because zoster can occasionally present as a single patch of rash and HSV can present with a zosteriform rash. In one study, vesicular fluid swabs were analysed for 110 individuals with vesicular eruptions presenting to a dermatology department.[36] All 65 individuals clinically diagnosed as zoster had their diagnoses confirmed by the laboratory tests, but nine (20%) of 45 patients who were clinically diagnosed as having HSV infection were found to have zoster. Eight of the nine 'HSV' patients had small patches of rash on the extremities or trigeminal dermatomes, and three had no pain. However, within two days the area of rash increased in four patients, developing a more typical zoster presentation. In a second study, 47 of 111 patients referred to a general hospital with clinical zoster underwent laboratory investigations.[44] Of these, six (13%) were found to have HSV infection.. All six had a 'dermatomal distribution' of rash, and 4/6 reported pain. It is unclear why only 47/111 patients underwent laboratory testing, and other researchers have suggested that some of these patients may have had atypical HSV infection due to undetected immunosuppression - the hospital was situated in an inner-city area with high prevalence of HIV infection.[45,46] Nevertheless, this study demonstrates that HSV can mimic zoster clinically.

As indicated above, laboratory methods can be used to distinguish zoster from other infections. Live VZV can be isolated from vesicle fluid for a limited period, but the sensitivity of diagnosis based on viral culture is low, ranging from 26-64%.[47] Polymerase chain reaction (PCR) allows sensitive (~100%) and specific detection of VZV-specific nucleic acids in vesicle fluid by amplifying conserved sequences of the viral genome.[48,49] The technique has also been used successfully to detect VZV DNA in crusted lesions.[49,50]

2.3 EPIDEMIOLOGY OF HERPES ZOSTER

Zoster is not a nationally notifiable disease in developed or developing countries, and there are limited surveillance data available. Some sentinel systems and studies in general practices have provided population-based descriptive data. Although zoster is thought to result from a loss of VZV-specific cell-mediated immunity, little is known about why this happens in individuals without underlying immunosuppression. The relatively few analytical epidemiological studies of zoster are described below.

2.3.1 Methods of identifying studies

Published articles on the descriptive and analytical epidemiology of zoster were identified by searching two electronic databases - Medline and Embase. No language restrictions were used.

Articles published up to mid-1998 were retrieved by searching for herpes zoster as a thesaurus term, with any of the following subheadings: 'epidemiology', 'aetiology', 'transmission', 'trends', 'statistics and numerical data'. Review articles on zoster were also identified, using a separate search. From mid-1998 onwards, monthly searches of the two databases were conducted, looking at all articles that indexed herpes zoster as a thesaurus term (all subheadings) or included 'zoster' as a free text term. Books on herpes viruses were identified by searching the databases of the London School of Hygiene and the British Library. Reference lists of all retrieved articles were examined, to identify publications and thesaurus terms not captured by the search strategy.

In addition to published articles, two major sources of information on the incidence of zoster in England and Wales were examined:

1. *Morbidity Statistics in General Practice (MSGP) studies:* these national studies of morbidity seen in general practice are run collaboratively by the Royal College of General Practitioners (RCGP) and the (then) Office of Population Censuses and Surveys (OPCS). Four studies have taken place – in 1955-56 (with 106 participating general practices), 1971-72 (43 practices), 1981-82 (48 practices) and 1991-92 (60 practices, providing for 502,493 patients).[23,51-53] For each study, participating practices provided details of every consultation within the study period, so that age- and sex-specific consultation rates for a wide range of diseases could be calculated. For the third and fourth study, new and 'first ever' episodes were reported separately, and thus incidence of specific conditions (including zoster) were available.

2. *The Royal College of General Practitioners (RCGP) Weekly Returns Service:* this service was established in 1967. Up to 91 participating practices have reported all episodes of illness by age and sex each week to the RCGP Research Unit, representing a population of more than 600,000 individuals.[54] Again, analysis by diagnosis provides data on incidence of specific conditions. Some of the Weekly Returns practices have also participated in the MSGP studies.

2.3.2 Descriptive epidemiology of zoster

Twenty-five reports that included population-based descriptive data on zoster were identified using the search strategy described above. Of these, five had no denominator information,[55-59] and two contained descriptive data entirely duplicated elsewhere.[5,60] The 18 reports which provided information on zoster incidence, together with data from the RCGP Weekly Returns Service and the MRSP surveys, are summarised in **Table 2.1** (overleaf). These comprised six

Table 2.1: Population-based studies of zoster incidence

Country[ref]	Date	Population	Case ascertainment	Diagnosis	Cases (n)	Incidence	Comments
USA[4]	1945-59	Inhabitants of Rochester, Minnesota (census data)	Database of diagnoses from OPD, hospitals, housecalls, nursing homes	Clinical case definition applied to medical records	590	$1.25/10^3$py	Rise in age-adjusted incidence from (1945-49) → (1955-59): 41% in men, 28% in women No evidence of seasonality
USA[17]	1960-81	Child (0-19yrs) inhabitants of Rochester, Minnesota (census data)	Database of diagnoses from OPD, hospitals, housecalls, nursing homes, death certificates, autopsies	Individual clinicians - questionable cases included if culture positive	173	$0.42/10^3$py	
USA[22]	1983-92	Family practice in Somersworth, New Hampshire	Records of all cases	Single GP	124	$3.3/10^3$/yr	No practice denominator: assumed 1/3 town serviced by practice No evidence of seasonality
USA[61]	1989-90	Cohort of 3206 independently living individuals in N.Carolina >64y old	Interview : zoster in last 3yrs	Self-reported	69	$7.1/10^3$/yr	Lifetime zoster: - urban 72% vs. rural 66% - black 26% vs. white 58%
USA[16]	1990-92	250,204 members of Harvard Community Health Plan:	Diagnoses from computerised medical records and claims files: emergency depts, ambulatory settings, hospitals, telephone consultations	Individual clinicians	1075	$2.15/10^3$ py	No trend in incidence over 2yrs
Canada[2]	1979-97	Individuals living in province of Manitoba	Physician billing claims	N/R	N/R	0-4y: $0.6/10^3$ py 5-14y: $1.2/10^3$ py 15-44: $1.9/10^3$ py 45-64: $4.2/10^3$ py 65+y: $8.1/10^3$ py	No evidence of seasonality
Scotland[20]	1947-48	Individuals attending GPs (15-20% of all practices) or hospitals in Edinburgh	Notified by GPs or hospital doctors	N/R	184	$2/10^3$/yr	Incidence calculated assuming practices saw 15-20% of all cases No evidence of seasonality No assoc. with: population density or household crowding/conditions
Scotland[18]	1948-55	General practice in Hawick: c.2400 individuals (2/3 suburban, 1/3 rural)	Records of all cases	N/R	81	$4.8/10^3$/yr	No difference in rural/suburban incidence
Scotland[19]	1972-73	8 general practices in Glasgow - c.36000 individuals	Notified by GPs	a) Individual GPs b) Paired sera	87	a) $2.4/10^3$/yr b) $2.2/10^3$/yr	

(key to abbreviations overleaf)

(continued overleaf)

Table 2.1 (continued): Population-based studies of zoster incidence

Country[ref]	Date	Population	Case ascertainment	Diagnosis	Cases (n)	Incidence	Comments
Scotland[21]	1955-85	General practice in Dumfriesshire 1850 patients	Records of all cases	N/R	151	$2.6/10^3$/yr	Increased incidence 1969-1982 Higher incidence May-Sept
England[34,71]	1947-62	General practice in Cirencester c.3500 patients	Records of all cases	N/R	192	$3.4/10^3$/yr	Higher incidence in summer+autumn
England/ Wales[53]	1981-82	48 general practices 332,270 patients (MSGP3)	Reporting of all diagnoses	Individual GPs	N/R	$3.7/10^3$py	
England/ Wales[23]	1991-92	60 general practices (88% urban) 502,493 patients (MSGP4)	Reporting of all diagnoses in face-to-face contacts	Individual GPs	N/R	$4.5/10^3$py	
England/ Wales[62]	1967-89	Up to 91 general practices 161729-239984 patients (RCGP)	Weekly returns of all diagnoses	Individual GPs	N/R	$3.2/10^3$/yr	No evidence of seasonality
England/ Wales[63]	1994-2001	Up to 85 general practices >570,000 patients (RCGP)	Weekly returns of all diagnoses	Individual GPs	N/R	$3.8/10^3$/yr[b]	No evidence of seasonality
Germany[64]	1992-93	Ansbach, Germany population c 40,000	All cases seen by GPs, dermatologists, paediatricians	Individual clinicians	152	$2.2/10^3$/yr	No evidence of seasonality
France[65]	1997-98	4635 GPs & 513 dermatologists throughout France	Notified by GPs	Individual clinicians	8103	$4.8/10^3$	
France[66]	1998	744 GPs (mostly urban)	Postal survey of GPs - cases seen in previous yr	Individual GPs	605	$3.2/10^3$	54% response rate by GPs
Italy[67]	1995	71 GPs throughout Italy 98,508 patients >15y old	Retrospective reporting by GPs: all cases seen in previous year	Individual GPs	408	$4.1/10^3$/yr	
Iceland[68,69]	1990-95	62 general practices (58% urban)	Notified by GPs + searches of computerised records	Individual GPs: researchers excluded if clinical history → 'unlikely to be zoster'	457	$2.0/10^3$py	No evidence of seasonality Incidence=$1.6/10^3$/yr in 2nd study of children/adolescents[69]
Netherlands[70]	1994-99	22 general practices in 6 areas c.49,000 patients	Searches of computerised records	Individual GPs	837	$3.4/10^3$/yr	Incidence standardised to Europe standard popl = $3.6/10^3$/yr

OPD = outpatient departments. N/R = not recorded; py=person years; MSGP = Morbidity statistics from general practice; RCGP = Royal College of General Practitioners

[a] Recalculated from data presented (reported as $3.7/10^3$/yr)

[b] See Figure 2.1 for annual incidences

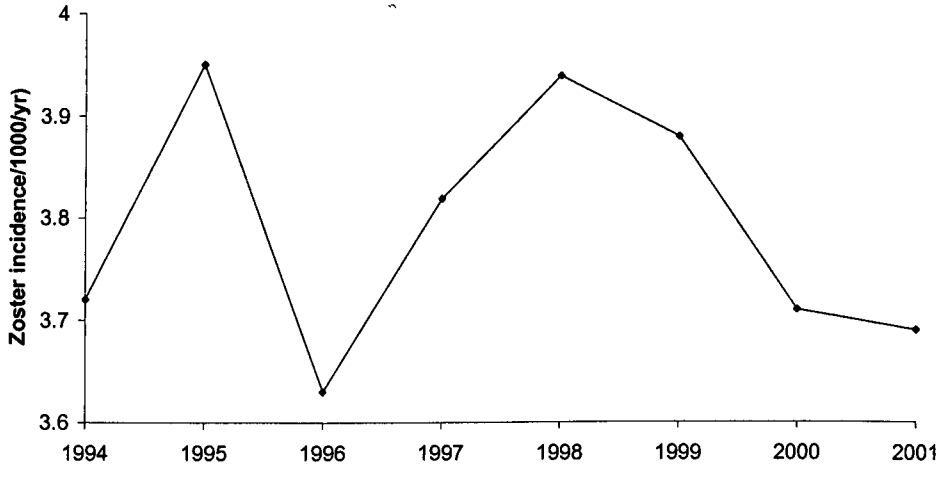
studies from North America,[2,4,16,17,22,61] nine from the United Kingdom (including two MSGP reports and two Weekly Returns summaries),[18-21,23,34,53,62,63] and seven reports from elsewhere in Europe including two analyses from the same study in Iceland.[64-70] In studies that included both younger and older individuals, overall incidences ranged from 1.3 - 4.8/10^3/year or per 10^3 person years. Within all these populations, there was a steep rise in zoster incidence with age; for example, in the 1991-92 MSGP Study, incidence ranged from 1.0/10^3 person years (in patients aged 0-4 years) to 13.0/10^3 person years (in patients aged 85 years or older).[23] The two studies that were restricted to children and to children/adolescents reported low incidences, of 0.42 and 1.6/10^3/year respectively, and the single study of elderly individuals (aged 65-104 years) reported an incidence of 7.1/10^3/year.[17,61,69]

Some studies reported no overall difference in zoster incidence by sex.[16,18,21,34] Others reported an excess of zoster incidence in females, but this may simply have reflected a larger proportion of women in the older (higher risk) age groups.[4,22,23,53,62] In the large US (Rochester) study, there was no significant overall difference in incidence between the sexes after adjusting for age, but a significantly lower incidence amongst females compared to males aged 35-44 years.[4] In the 1991-92 MSGP study, age-specific incidence was higher amongst females aged 45-64 years (7.1 vs. 5.1/10^3py) and 65-74 years (12.2 vs. 9.0/10^3py), but less marked in other age groups – the statistical significance of these differences was not reported, and there may have been residual confounding within the age bands. In the Iceland study, age-standardised incidence of zoster amongst the 60+ years age group was higher amongst women (5.6 vs. 3.6/10^3 py).[68] Only one study reported zoster incidence by ethnicity - the risk was significantly lower amongst elderly individuals of black ethnicity compared to white ethnicity after eight years of follow-up (1.4% versus 3.4%, $p<0.001$).[61]

Seasonality of zoster incidence was not found in most of the population-based studies.[2,4,20,22,56,57,62,64,68] However one Scottish and one English study reported higher incidence of zoster in summer and autumn.[21,71] Clinic-based studies have also reported seasonality of zoster incidence, amongst physiotherapy patients in England (increased incidence in summer), dermatology patients in Sweden (summer), dermatology patients in the Netherlands (summer/autumn), emergency department attenders in Italy (summer), and renal transplant recipients in the Netherlands (summer).[72-76] Similarly, higher zoster incidence was found in the warmer, drier months amongst dermatology patients in Indonesia.[74] Caution is needed in interpreting the results from clinic-based data, as case ascertainment may be incomplete, especially during the colder months.

Some of the variation between studies in zoster incidence may result from differences in methodology or in the age structure of the populations, or secular changes. In the Rochester study, there was a 41% age-adjusted rise in males and a 28% rise in females in 1955-59 compared with 1945-49[4] The 1990-92 study of a similar US population showed a 64% rise in zoster incidence compared to the Rochester study, after standardising for age to the same 1970 US white population.[16] The proportion of individuals documented as having cancer were similar in the two studies (6.4% vs. 6%), although 4.5% of individuals in the later study were also known to be human immunodeficiency virus (HIV) positive and the proportion of individuals on immunosuppressive therapies was not reported. It is also possible that the later study had more complete ascertainment of cases, as a result of free access to health care. In the UK, increase in overall zoster incidence was not found in the aggregated data of the first 23 years reporting by the RCGP Weekly Returns Service, or from the last eight years of available data (**Figure 2.1**).[63] However, the RCGP reported a slight increase in the number of cases in 1998 amongst those aged ≥65 years compared to the previous ten-year average.[62,77] In the MSGP studies, there was an increase in zoster incidence from $3.7/10^3$/year (MSGP3) to $4.5/10^3$ py (MSGP4). This increased incidence was also seen for each of the individual age groups 25-44 years, 45-64 years and 65-74 years (older ages were grouped differently, so could not be compared). Comparisons with earlier MSGP data are limited, as the first two studies did not document new episodes of disease separately, but included prevalent cases consulting in the study year which arose in previous years.[52,78] Nevertheless, there was an increase in these 'patient consulting rates' (prevalent cases and new cases) in successive MSGP studies, from 3.5/1000 in 1955/56[78] to 4.9/1000 person-years in 1991/92.[23]

Figure 2.1: Annual incidence of herpes zoster (per 1000) in England & Wales (RCGP data)



2.3.3 Determinants of zoster

If VZV infection is maintained in the latent state by specific cell-mediated immunity, it follows that factors that predispose to loss of immunity may increase the risk of zoster. This may be loss of VZV immunity alone, or generalised diminution in cell-mediated immune responses. Both of these scenarios are discussed below.

2.3.3.1 Loss of specific immunity to VZV

Ageing: as described above, the risk of zoster increases sharply with age. Specific cell-mediated immunity to VZV is decreased in elderly individuals, as measured by specific skin test and by examining viral-specific cytotoxic T lymphocytes.[9,10,31,79] This may be due to the waning of specific immunity with increasing time from primary infection, or may be part of the generalised decay in cell-mediated immunity that occurs with age (discussed in Section 2.3.3.2, below).

Age at primary infection: if zoster results from waning of specific immunity, then individuals who acquire varicella later in life may be at decreased risk of zoster. No studies were identified that specifically examined this hypothesis, but it has been suggested as an explanation for the finding that on multivariable analysis in one study, elderly individuals of black ethnicity were at approximately one third the risk of zoster compared to those of white ethnicity (RR=0.35, 95%CI=0.24-0.51).[80,81] The average age at infection for varicella differs in temperate and tropical regions. It is typically a disease of childhood in temperate countries, with approximately 90% of children immune by the age of ten years.[82-85] However, in some tropical countries the average age at infection may be delayed to adolescence or adulthood, as has been demonstrated by serosurveys of residents or immigrants from the Caribbean, Southern India, Sri Lanka, and parts of South East Asia and Central America.[86-93] However, some tropical populations appear to have early onset of varicella, as shown in studies of Northern Australians, urban Brazilian children, rural Bolivians, Calcutta slum residents and Japanese children.[94-98] Hypotheses for delayed age at varicella include inactivation of virus and reduced transmission in hot, humid conditions, reduced mixing patterns in tropical rural areas, cross-immunity between HSV and VZV and competition with other viruses.[86,92,97,99,100]

In contrast to late age at varicella, acquisition of varicella in infancy or *in utero* may increase the risk of zoster. Guess *et al* demonstrated that children who acquired varicella in the first year of life were at nearly three times the risk of zoster in childhood or adolescence (RR=2.8, 95%CI=1.6-4.7).[17] In a study of 849 children, Baba *et al* reported nine cases of zoster, all of

whom acquired varicella in the first year of life.[101] Zoster incidence was significantly higher in the children who developed varicella before two months of age compared to those who were infected between 2-11 months. Enders *et* al reported that 10/1291 infants born to mothers who had varicella during pregnancy developed zoster during infancy or early childhood; maternal varicella between 13-24 weeks and 25-36 weeks of pregnancy were associated with childhood zoster risks of 0.8% and 1.7% respectively.[102] It is possible that the immature immune system of the infant or foetus is less able to establish and maintain viral latency.

Exogenous boosting of specific immunity: Hope-Simpson hypothesised that exogenous exposure to infectious cases of varicella or zoster might boost specific immunity and therefore decrease the risk of zoster in latently infected individuals.[34] Mothers of children with varicella experience VZV cell-mediated immune boosting, and the significantly lower zoster incidence amongst women aged 35-44 years in the Rochester study could reflect increased contacts with children (and therefore with varicella).[4,103] If regular contact with varicella decreases the risk of zoster, the following might be expected:

1. *Seasonality of zoster is inversely correlated with seasonality of varicella:* the lack of seasonality in zoster incidence in most population-based studies does not support this hypothesis. However, the handful of studies described in Section 2.3.2 that reported higher incidence of zoster cases in the summer months (when varicella is at its lowest) do support the theory of a protective effect of varicella. A further clinic-based study from Japan reported that monthly and annual incidence of zoster showed an inverse relationship with varicella incidence, although this did not reach statistical significance.[104] It is possible that the average duration of immune boosting following varicella contacts may be long relative to the yearly epidemic time scale of varicella, in which case simple inverse correlations may not be seen. Garnett and Grenfell attempted to clarify the relationship between varicella and zoster incidence using time series analysis of RCGP data.[105] There was no association between the two diseases at the weekly level, suggesting that varicella incidence had no immediate effect on zoster incidence. However at the annual level, an increase in varicella incidence among children under five years old was accompanied by a significant decrease in zoster incidence among individuals aged 15-44 years. This suggests that increased varicella among young children could exert a protective effect against zoster in the young adults exposed to them.

2. *Zoster incidence is decreased in areas of high population mixing:* in two GP-based studies, there was no association between zoster incidence and rural/suburban residence or population density/household crowding.[18,20] In the US study of elderly individuals,

lifetime zoster was not associated with urban residence on multivariable analysis (OR=1.01, 95% CI=0.80-1.26).[61] However, none of these studies provided information on mixing patterns with children, who are the sub-population most likely to have varicella.

3. *Zoster incidence is low amongst individuals with occupational contacts with varicella:* Two studies have investigated this hypothesis. Responses to a US postal questionnaire by 1109 paediatricians, 1984 dermatologists and 462 psychiatrists showed that paediatricians had most contacts with VZV-infected patients and were significantly less likely to have developed zoster, but the results may have been influenced by the <40% response rate.[106] Similarly, 34 (9.1%) of 352 Japanese paediatricians and family practitioners who responded to a questionnaire reported a history of zoster, and this was estimated to be 50-87% lower than the age-specific incidences in the general population.[107]

There is little other analytical epidemiological information on the association between zoster and varicella contacts. A study of 511 leukaemic children vaccinated against VZV showed that subsequent household exposure to varicella and further doses of vaccine were both highly protective against zoster.[108] However it is unclear whether exogenous exposure protects against zoster in immunocompetent adults.

2.3.3.2 Generalised loss of cell-mediated immunity

The failure to maintain VZV latency may also occur because of a generalised diminution in cell-mediated immunity. This may occur under the following conditions:

Immunosuppressive conditions/therapies: individuals with diminished cell-mediated immunity due to pre-existing cancers, autoimmune disorders, HIV infection and other conditions necessitating immunosuppressive therapies are at higher risk of zoster, with incidences ranging from 25.0 – 91.5/10^3 person-years.[13-15,109-119] Immunosuppressed individuals constituted 1-11% of cases in population-based studies.[4,16-22] In studies of young African populations, zoster has a 85-95% positive predictive value for underlying HIV infection.[120-124] In contrast, incident zoster may not be a good indicator of occult cancer. In the Rochester study, subsequent incidence of cancer was not significantly higher amongst individuals with zoster after 9389 person years of follow up compared to local residents without zoster (RR=1.1, 95% CI=0.9-1.3).[125] Similarly, Schmader *et al* reported that in a cohort study of elderly individuals, those with a history of cancer were not at increased risk of zoster after eight years of follow up (adjusted RR= 1.03, 95%CI=0.58-1.80), although individuals who self-reported their health as 'excellent' were at half the risk of subsequent zoster (adjusted RR=0.51, 95%CI=0.27-0.95).[80]

Ageing: the increasing risk of zoster in the elderly may reflect the generalised decay in cell mediated immunity which occurs as part of the ageing process.[126-131] Immunosenescence is a likely contributor to the increased susceptibility to infections, malignancies and autoimmune disorders in the elderly.[132] However, the age at onset and degree of cell-mediated immune impairment associated with ageing varies widely, and some older individuals have immune responses similar to those of much younger individuals.[133,134] If zoster occurs as a result of immunosenescence, then investigation of risk factors for zoster may shed light on the determinants of loss of immune function with age.

Psychological stress: stress affects a number of neuroendocrine functions and this can result in cell-mediated immune suppression.[135] Both acute and chronic stress events may be associated with immune dysfunction. For example, individuals in two studies who were suffering acute stress from bereavement had a significant reduction in T-cell function one to two months after bereavement compared to non-bereaved controls or pre-bereavement function,[136,137] and a non-significant reduction for up to fourteen months.[137] Students experiencing acute stress prior to examinations have been shown to have impaired lymphoproliferation to mitogens or CD4+/CD8+ T-cell ratios.[138,139] Carers of chronically ill patients have lower proliferative responses to mitogens and lower humoral and cell-mediated immune responses to vaccination compared to non-caregivers.[140,141] Irwin *et al* demonstrated that 11 adults with major depression had lower VZV-specific cellular immunity compared to age/sex matched controls without depression.[142] In contrast to the above findings, short-term stress can sometimes result in enhanced cell-mediated immune responses.[143,144] It is likely that the effect of stress on the immune system depends on the severity and duration of the stress, and on individuals' defence and coping mechanisms.[145,146]

Stress also may increase susceptibility to infectious disease, including reactivation of latent herpes virus infections.[147-152] Two studies have investigated the effect of stress on risk of zoster. In the first, 101 elderly individuals with recent zoster and 101 age-matched controls were asked about stressful life events in the year preceding zoster onset in the cases, and whether they perceived these events as negative.[153] Cases experienced a significantly higher number of events in the six months before rash compared to controls (mean 2.64 vs. 1.82 events, p=0.008). In the two months before rash, there were no significant differences in the overall number of events but cases were significantly more likely to have experienced negatively-perceived events (OR=2.60, 95%CI=1.13-6.27). However, these differences could have been due to recall bias. A second study of the same elderly population recorded 167 cases of zoster amongst 2568 individuals after eight years of follow up.[80] Negatively-perceived life events were weakly associated with risk of zoster on multivariable analysis (RR=1.38,

95%CI=0.96-1.97, p=0.078), but social support variables (such as presence of a confidant) were not associated with zoster risk. An incidental finding in this study was that cigarette smoking was associated with significant protection against zoster (adjusted RR=0.47, 95%CI=0.25-0.89).

Chemical exposures: certain pesticides, volatile organic substances (e.g. toluene, benzene) and metals such as arsenic and mercury can suppress cell-mediated immunity. Early reports mentioned arsenic as a risk factor for zoster.[34,74,154] A cross-sectional study of 900 individuals aged between 18-40 years who lived within 2.5 miles of pesticide dump sites (repositories for organochlorines, volatile solvents and metals) in Aberdeen (North Carolina) found that they were twice as likely to report a history of zoster at telephone interview compared to individuals from neighbouring communities (multivariable RR=2.1, 95%CI=1.0-4.3).[155] However, the temporal sequence of residence in the area and zoster was not ascertained. Older individuals in the study were not at increased risk of zoster, and the authors suggested that they may have had lower exposure levels to the chemicals, due to fewer outdoor recreational activities than younger individuals.

2.3.3.3 Other determinants of zoster

Mechanical trauma: this was associated with the development of zoster in 1-5% of cases in population based studies.[4,19,34] Recent surgery and/or radiotherapy to the affected dermatome was also reported in 1.7% of cases in the Rochester study.[4] Juel-Jensen suggested that local trauma preceded the onset of zoster in 38% of his series of 100 cases.[156] However none of these studies employed controls for the history of trauma, which may be common in elderly populations.

VZV contacts precipitating zoster: a few authors of case reports[20,157-164] and small clusters of zoster[13,109,165-168] have suggested that zoster-zoster or varicella-zoster transmission may occur. None of these studies used control subjects to estimate the population exposure to varicella or zoster, or carried out molecular analyses to determine the similarity of viral strains in the cases. Most zoster cases in clusters were immunosuppressed, and some cases with atypical presentations may have had second episodes of varicella rather than zoster.[13] In one cluster of seven employees who developed zoster over a three-month period, three of the seven cases had bilateral lesions, which is rare in zoster.[166] However, four of the cases had measurable VZV-specific IgM, compared with none of the 22 employees who did not develop zoster. The only (unpublished) study which has carried out molecular analyses found that each of the five patients involved was infected with a different viral strain.[165]

Genetic susceptibility: a recent study demonstrated that a significantly higher proportion (53%) of 60 immunocompetent patients with zoster carried the ATA haplotype at the promoter region of the interleukin-10 (IL-10) gene, compared to 152 (38%) of 400 blood donors.[169] It is unclear whether this haplotype is associated with diminished or enhanced IL-10 production capacity.[170,171] High circulating IL-10 levels could increase the risk of VZV reactivation by down-regulating cytokines of the T-helper cell-1 (TH_1) subset, as discussed in Section 2.3.4.1, below. Also, if polymorphisms in the IL-10 gene are associated with general susceptibility to a range of infections or other immune-mediated disorders (in addition to VZV reactivation), then voluntary blood donors (who are essentially healthy) may not have been a suitable control group.

2.3.4 Other putative risk factors for zoster

As outlined in Section 2.3.3.2, older individuals are at increased risk of zoster, but little is known about the determinants of immunosenescence. Two exposures that cause diminution in cell-mediated immune functioning, and which might be risk factors for age-related immune decay (and therefore zoster) are ultraviolet radiation (UVR) and insufficient dietary micronutrient intake. These putative risk factors are discussed below.

2.3.4.1 Ultraviolet radiation exposure

Sunlight comprises both UVB (wavelength: 280-320nm) and UVA (wavelength: 320-400nm).[172] Absorption of UVR by proteins and nucleic acids in the epidermis is mostly restricted to shorter UVR wavelengths, and so most of the health effects of UVR result from UVB exposure. Stratospheric ozone partially absorbs UVB and prevents it from reaching the Earth's surface.[172] It follows that the recent reduction in the atmospheric ozone layer together with changes in human sun-seeking behaviour has resulted in increased exposure to UVB.

Acute UVR exposure has been widely shown to diminish cell-mediated immune responses both locally (at the exposed site) and systemically (at non-exposed sites).[173] The mechanisms involved have not been fully elucidated, but are thought to include the following steps. Firstly, UVR is absorbed into photoreceptor molecules in keratinocytes. The main photoreceptors are thought to be DNA and urocanic acid. Following UVR exposure, pyrimidine dimers are formed in keratinocyte DNA and trans-urocanic acid is photoisomerised to the cis-isomer, and both these events have been implicated in immunosuppression.[174-176] Damaged keratinocytes 1) produce PGE_2 and IL-10, which decrease antigen presentation, increase IL-4 production and down-regulate TH_1 cytokine production, resulting in local and systemic suppression of cell-

mediated immunity,[177,178] and 2) up-regulate IL-1β and tumour necrosis factor-α (TNF-α), causing migration of Langerhans cells from the epidermis.[179,180] UVR-damaged Langerhans cells migrate to draining lymph nodes, where their impaired antigen-presenting function results in diminished proliferation of TH_1 cells.[181-183] Langerhans cells are replaced in the epidermis by other antigen-presenting cells (macrophages), which preferentially activate suppressor-inducer T lymphocytes, and thus contribute to immune suppression.[184-186]

Studies of UVR and cell-mediated immune responses: Studies of the effects of UVR on human populations often measure UVR exposure in terms of the minimal erythemal dose (MED). This can be defined as the minimum dose of UVR needed to produce a just perceptible erythema in unacclimatised skin 24 hours after exposure.[187] The amount of UVR exposure needed to produce one MED varies between individuals, but estimates for 'average' MED have been made - for example, approximately 20 minutes exposure to sunlight at latitude 40^0 at midday in June will result in one MED.[188]

Studies of the effect of UVB exposure (alone or combined with UVA exposure) on cell-mediated immune responses in humans are summarised in **Table 2.2** (overleaf).[185,189-209] Most enrolled small numbers of (often young) individuals. Of the three studies of acute exposure to varying doses of natural sunlight, two reported immunosuppression in terms of lower levels of circulating $CD4^+$ (T helper) cells, increased levels of $CD8^+$ cells, and (in one study) increased suppressor T-cell activity.[189,190] Both studies were of individuals with previously low UVR exposures. The third study reported immunostimulation in the form of increased natural killer (NK) cell counts following summer holiday UVR exposure, but participants may have had higher baseline UVR exposures.[191]

Other studies in **Table 2.2** used artificial light sources, either UVA/UVB lamps that simulated sunlight exposure, pure UVB lamps, or predominantly UVA-emitting lamps that simulated solarium exposure. Most studies that applied ≥1 MED dose of UVR demonstrated local suppression of cell-mediated immunity lasting from a few hours to a few weeks, with impaired responses to contact allergens[192-200] or to pathogen antigens[195,201] applied to the irradiated site, and increased local suppressor T-cell responses.[185] Systemic effects were also reported, including decreased responses to antigens or allergens applied to non-irradiated sites,[197,200,202,203] a drop in numbers of circulating T-cells, $CD4^+$ T-helper cell subsets or NK cells,[192,198,202,204] and increased numbers of circulating $CD8^+$ cells or T-suppressor cell activity.[198,204] In the seven studies that looked at acute low-dose UVR exposures (<1 MED/day), local immunosuppression was seen in four studies,[193,197,199,205] and systemic

Table 2.2: Studies of the effects of acute ultraviolet B radiation exposure (with/without ultraviolet A radiation) on cell-mediated immunity in human populations

	STUDY POPULATION / EXPOSURE				RESULTS IN IRRADIATED GROUP	
Study [ref]	**UV exposure / dose**	**Irradiated Group**	**Non-irradiated comparison**	**Follow up**	**Local responses**	**Systemic responses**
Hersey[189]	Sunlight: whole-body exposure 30min/day for 12d (1.2-1.7 MED/day)	12 volunteers mean age: 32.1y ± 6.2y	a) Pre-irradiation levels b) 13 age matched controls	2w	N/D	↓ T cells ↓ $CD4^+$ ↑ $CD8^+$ Slight ↓NK ↑ T_s activity
Falkenbach[190]	Sunlight: winter holiday exposure for 7-57d (mean daily UVB=985.6mJ/cm^2)	32 individuals → holiday (lat: 35°N-40°S) mean age: 31y ± 10y	Pre-holiday levels	≤1w	N/D	↓ $CD4^+$: $CD8^+$
Kanariou[191]	Sunlight: summer holiday exposure in Greece for ±3wks	12 individuals→ holiday median age: 21y (10-45y)	Pre-holiday levels	≤1w	N/D	Changes to $CD3^+$, $CD4^+$, $CD8^+$ n/s ↑NK
Baadsgaard[185]	Sunlamp (UVB/UVA): single 4MED dose to forearm	Volunteers	Non-irradiated skin	3d	↑ T_s responses	N/D
Cestari[201]	UVB/UVA: buttock exposure to 2MED every 4d for 20 days	29 lepromin+ve contacts of leprosy patients (18-62y)	Non-irradiated skin	7d	↓DTH to lepromin ↓ %$CD4^+$ cells in granulomas	N/D
Di Nuzzo[209]	Sunlamp (UVB/UVA): lower back exposures to 1, 2 & 4 MED	5 volunteers mean age: 27.8y (18-47y)	Non-irradiated skin	7d	1-2d: ↓ $CD3^+$ 7d: ↓ $CD8^+$; ↑ $CD4^+$	N/D
Kelly[193,200]	Xenon arc (UVB/UVA): buttock exposure to 0.25, 0.5, 1, 2 or 3 MED	77 volunteers	16 volunteers	3w	↓DNCB reactions[193] skin types I/II: ↓ at all UV doses skin types III/IV: ↓ at ≥IMED	↓ DPCP response in unexposed skin in 10/12[200]
Morrison[192]	Sunlamp (UVB): single whole-body exposure to 1.5 or 3 MED	10 fair-skinned volunteers mean age: 26y (22-40y)	Pre-irradiation levels	72hr	3 MED: ↓ response to PHA 1.5 MED: change n/s	3 MED: ↓ T-cells (normal by 72h) 1.5 MED: change n/s
Kalimo[194]	Sunlamp (UVB) single dose to forearm: strong erythema (n=9), mild erythema (2), no reaction (2)	13 patients with contact allergies mean age: 49y (23-82y)	Non-irradiated skin	4d	↓ DTH to 19 out of 25 contact allergens	N/D
Rasanen[195]	UVB bulbs: single dose to abdominal skin (2-4 MED)	11 dermatology patients mean age: 38y (28-52y)	a) Non-irradiated skin b)18 dermatology controls	0-7d	↓ LP to PPD, HSV-1, ConA (Function restored in 3-7d)	N/D

(contd. overleaf)

N/D = not done; n/s = not significant; hr=hours; d = days; w = weeks
UVR = ultraviolet radiation (UVA or UVB); MED = minimal erythemal dose; skin type I/II=sun sensitive, tans poorly; skin type III/IV=sun tolerant, tans easily
DNCB = dinitrochlorobenzene; DPCP = diphenylcyclopropenone; ConA = concanavalinA; PWM = pokeweed mitogen; PHA = phytohaemagglutinin;
PPD = purified protein derivative; Ni = nickel; HBV = hepatitis B virus; HBsAg=hepatitis B surface antigen; HSV = herpes simplex virus; ag= antigen(s)
$CD3^+$ = pan-T cells; $CD4^+$ = helper T-cells; $CD8^+$ = cytotoxic / suppressor T-cells; T_s = suppressor T-cells; NK=natural killer cells

Table 2.2 (continued): Studies of the effects of acute ultraviolet B radiation with/without ultraviolet A radiation on cell-mediated immunity in human populations

Study [ref]	STUDY POPULATION / EXPOSURE				RESULTS IN IRRADIATED GROUP	
	UV exposure / dose	Irradiated Group	Non-irradiated comparison	Follow up	Local responses	Systemic responses
Miura[203]	Lightbox (UVB): 90% body exposure to 1MED	6 individuals with latent HSV infection (34-57y)	a) Pre-irradiation levels b) 1 HSV infected control	9-17d	N/D	↓LP to HSV + PHA (lasted ≥9d)
Yoshikawa[196]	UVB: buttock exposure to 1.44mJ/cm^2/day for 4d	12 skin cancer patients 34 healthy volunteers	6 healthy volunteers	0d	DNCB response in 100% controls, 60% healthy (UV), 8% cancer (UV)	N/D
Sleijffers[202]	Cabinet (UVB): ± whole body exposure to 1MED/day for 5d before vaccination	97 volunteers receiving HBV vaccination (age: 19-52y)	94 non UVB-exposed HBV vaccinees	60d		↓ DPCP response in unexposed skin ↓NK activity LP to PHA, PWM, HBsAg unaffected
Cooper[197]	Sunlamp (UVB): buttock exposure 0.75MED or 2MED daily for 4d, or single dose of 4MED	42 white volunteers	22 controls	0-2d	↓DNCB reactions in all UV groups (dose-response)	4MED: ↓ DPCP responses (other groups n/s)
Hersey[204]	Solarium (mostly UVA): whole-body exposure 30min/day for 12d (± 1MED/day)	22 volunteers 11/22 wore sunscreen	a) Pre-irradiation levels b)12 controls: sunscreen	3w	↓ DTH (Multitest) – n/s DNCB response unaffected	↓ T cells; ↓ NK activity (normal by 3w) 11 'sunscreen': ↑%CD8$^+$, ↓CD4$^+$:CD8$^+$
Hersey[198]	Solarium (mostly UVA): whole-body exposure 30min/day for 12d	18 untanned volunteers mean age: 30.5y ± 10y	a) Pre-irradiation levels b)13 age matched controls	2w	↓ DNCB response at 2 days	↓ lymphocytes (remained ↓ at 2w) ↓ CD4$^+$; ↑ CD8$^+$; ↑ T_s activity at 2d ↓ NK activity at 2wks
Sjovall[205]	Sunlamp (UVB): ± whole body exposure to <1MED 4x/w for 3w	9 patients with Ni allergies mean age: 34 y (22-44y)	a) Prior responses b) Non-irradiated skin	0d	↓ DTH to Ni	↓ DTH to Ni in unexposed skin
Damian[199]	Sunlamps to >1 site on back. a) UVB+UVA or b) UVB, for 2d-4w Mean dose = 0.6 MED/day	54 volunteers with Ni allergies mean ages: 26-34y	Non-irradiated skin	up to 4w	Max ↓ DTH to Ni at 2d (↓ for 3w) Effect of UVA/UVB > UVB only	DTH to Ni in adjacent skin unaffected
Gilmour[208]	Cabinet (UVB): ?whole body exposure to <1MED 3x/w for 4-6wk	17 psoriasis patients mean age: 37.5y (±3.1y)	a) Pre-irradiation levels b) 15 psoriasis patients	4w	N/D	Slight ↓ CD8$^+$, CD4$^+$ – n/s No change in LP to HSV, ConA
Matsuoka[207]	Chamber (UVB): whole body exposure to single dose of <1MED	20 women: 10 white, 10 black (age: 21-59)	Pre-irradiation levels	9hr	N/D	CD3$^+$ & CD8$^+$ unchanged Whites:↑ CD4$^+$, Blacks: ↑ NK activity
McGrath[206]	Sunlamp (60% UVB): total body exposure to 0.5 MED, 1MED 1d later	15 white volunteers	Pre-irradiation levels	2d	N/D	0.5 MED: ↓% CD8$^+$, ↑CD4$^+$: CD8$^+$ + 1MED: levels normalised

Abbreviations: see previous page

immunosuppression in one study.[205] However, two studies reported transiently increased immune responses,[206,207] and one study reported no change in T-cell subsets or lymphoproliferation to pathogen or mitogen antigens.[208]

The finding that not all individuals experience diminished responses to contact allergens following UVR exposure has led to the hypothesis that individuals may be 'resistant' or 'susceptible' to the immunosuppressive effects of acute UVR.[196,210] There is conflicting evidence that susceptibility is mediated by skin type (tendency to burn, ability to tan, and/or skin colour).[193,207,211-213] When responses are stratified by UVR dose, it appears that individuals who are sun-tolerant and tan easily may be resistant to the immunosuppressive effects of low-dose (≤1MED) UVR,[193,207,212] but that individuals of all skin types may be susceptible to higher UVR doses.[193,200,211,212]

Little is known about the effects of chronic UVR exposure on cell-mediated immunity. In animals, decreases in DTH reactions were reversed after 20 UVR exposures, suggesting adaptation, although suppression of tumour rejection was maintained.[214] In humans, chronically UVR-exposed skin has fewer Langerhans cells and reduced sensitivity to contact allergens compared with non-exposed skin.[215,216] A study of skin cancer patients reported that those with high UVR exposure in the previous 18 months had lower ratios of $CD4^+$:$CD8^+$ T cells compared with patients with low exposure levels.[217] In contrast, in two cohorts of HIV-positive men there was no significant correlation between UVR exposure since becoming HIV infected (up to ten years previously or in the last two years) and the rate of $CD4^+$ T-cell decline.[218,219] However, measurement of past UVR exposure in one of the cohorts was imprecise, being based on answers to simple questions such as outdoor occupation, beach vacations and outdoor hobbies, and in the second cohort only a small number of participants (n=73) completed the questionnaire.

Effects of UVR on infections: In animals, UVR exposure decreases the immune response to a variety of infectious agents, including HSV, *Leishmania*, *Candida*, *Trichinella*, *Schistosoma*, *Listeria* and *Mycobacterium sp.*[220-226] It also promotes HIV gene expression,[227] and increases pathogen load or lesion severity in experimentally infected animals.[220,224-226,228,229] Researchers have attempted to use these data to make risk assessments of the effect of UVR on susceptibility to and severity of infections in humans, taking into account differences between animals and humans in sensitivity to UVR-mediated immunosuppression. For example, it has been estimated from animal models that 92 minutes of UVR exposure received over seven

days at noon in July at 40^0N may suppress cell-mediated immune responses to *Listeria monocytogenes* in humans by 50%.[76]

The effect of UVR on infections in humans has not been extensively studied. As outlined in **Table 2.2**, acute UVR exposure can diminish cell-mediated responses to pathogen antigens,[195,201,203] although this was not demonstrated in all studies.[202] Variation in responses to vaccination by latitude has also been demonstrated. For example, the efficacy of Bacillus Calmette-Guérin (BCG) vaccine against tuberculosis is greater at sites further from the Equator, where ambient UVR exposure is lower.[230] However, this may be due to factors other than UVR exposure. Another approach is to measure seasonality in responses to vaccination. In one study, Dutch students vaccinated against hepatitis B virus had lower mean antibody titres after the first vaccine dose if they were vaccinated in the summer, although there was no significant difference in titres by season of vaccination after the third dose.[76] Secular trends can also be investigated. Á pilot project was instigated in Chile to investigate the effect of increased UVR exposure due to thinning of the ozone layer.[231] Review of ophthalmologic and dermatologic records did not reveal any increase in consultations for skin infections during periods of ozone depletion, although the annual ambient UVB exposure during these times only increased by 1%.

Two recent studies have attempted to relate personal UVR exposure to susceptibility to infections. Amongst a cohort of children aged one year, exposure to UVR in the previous six weeks was ascertained by questionnaire.[76,232] On multivariable analysis, children with low recent UVR exposure had a significantly higher incidence of symptoms suggesting upper respiratory tract infection. The authors suggested that this unexpected finding might be because children with low UVR exposure were more heavily exposed to respiratory pathogens in indoor settings. However, a slight increased incidence of symptoms was also found for children who had been sunburned. In a second cohort of renal transplant patients there was no overall correlation between cumulative lifetime UVR exposure (ascertained by questionnaire) and frequency of infections.[76,233]

Exposure to UVR has also been shown to increase reactivation of latent infections. Herpes simplex infection can be reactivated in humans following UVR exposure of 3-6 MED.[234-237] Descriptive studies (summarised earlier in this Chapter) that have reported increased incidence of zoster in summer support the hypothesis that short-term UVR exposure may increase the risk of zoster. However, the effect of UVR exposure on reactivation of VZV has not been studied directly.

2.3.4.2 Micronutrient intake

The cells of the immune system are dependent on micronutrients for their functional integrity. For example, vitamin A is needed for lymphocyte proliferation, in addition having a major role in humoral and innate immune responses.[238,239] Vitamin B_6 is involved in the synthesis and metabolism of nucleic acids and proteins (and so is important for lymphocyte proliferation), and also has a role in the production of thymulin, a hormone that induces T-cell maturation in the thymus and regulates T-cell function.[240] Vitamin C prevents DNA damage in lymphocytes by limiting free radical formation, decreases T cell death, and up-regulates natural killer cell activity.[241,242] Vitamin E is also an antioxidant, protects the cell membranes of immune cells from damage, enhances T-cell function by inhibiting PGE_2 production by macrophages, and facilitates lymphocyte maturation and proliferation via IL-2 production.[243] Folic acid is a coenzyme for DNA synthesis in immune cells, and thus influences lymphocyte production and proliferation.[244] Zinc is an essential cofactor for more than 300 enzymes and hormones involved in cellular function (including thymulin), and is involved in bone marrow T-cell precursor formation and production of TH_1 cytokines.[245-247] Iron plays a key role in lymphocyte maturation, differentiation and proliferation.[248] Multiple deficiencies of these nutrients occur increasingly with age, particularly in institutionalised individuals.[249-254] Micronutrient deficiencies in the elderly are associated with diminished cell-mediated immunity (demonstrated by lower percentages of CD4+ T-cells, impaired lymphocyte proliferation and reduced DTH responses to skin test antigens), and increased susceptibility to infections.[255-262] The heterogeneity in cell-mediated immune decline in the elderly may therefore be partly explained by differences in micronutrient intake.

Placebo-controlled randomised controlled trials have been instigated to investigate whether supplementation with single or multiple micronutrients improves cell-mediated immune functioning in the elderly (**Table 2.3**, overleaf).[259,263-272] Many of these trials enrolled small numbers of participants. Increases in some immune parameters were reported amongst supplemented individuals in most studies, but these increases were not always significantly higher than increases in the placebo group. Of the four trials that used multivitamin/mineral preparations, three (all of which supplemented individuals for 12 months) reported significant increases in some or all of the cell-mediated immune parameters studied compared to individuals receiving placebo,[269,270,272] but one (which supplemented individuals for ten weeks) found no difference between groups in T-cell, T-cell subset or NK cell counts, or in lymphocyte function.[271] Differences in findings may have been due to variation in the duration and dosage of micronutrient intake and in the baseline nutritional and immune status of the participants. The remaining studies used single or simple combinations of micronutrients. The

Table 2.3: Placebo-controlled randomised controlled trials of the effect of micronutrient supplements on cell-mediated immune parameters in elderly populations

Study[ref]	Study population	Micronutrient intervention	Daily dose	Duration	Effects on cell-mediated immune parameters (supplemented vs. placebo)
Bogden[263]	103 independently living healthy individuals aged 60-89y	Zinc+ multivitamin/mineral (Placebo: multivitamin/mineral)	Zinc: 15mg or 100mg	3m	No significant differences in DTH (Multitest) or LP
Bogden[264]	63 independently living healthy individuals aged 60-89y[c]	Zinc+ multivitamin/mineral (Placebo: multivitamin/mineral)	Zinc: 15mg or 100mg	12m	↓DTH (Multitest) – especially 100mg group ↑NK cells; No significant differences in LP
Kennes[265]	20 healthy individuals aged >70y	Vit C	500mg injection	1m	↑DTH, ↑LP } Unclear if significantly > placebo
Meydani[266]	32 healthy individuals aged ≥65y	Vit E	800iu	30d	↑DTH (Multitest); ↑IL-2 ↑LP to ConA (not PHA or SAC)
Meydani[267]	78 independently living healthy individuals aged ≥65y	Vit E	60mg, 200mg or 800mg	4m	↑DTH (Multitest) - significant for 200mg No difference in T cells
Pallast[268]	157 independently living healthy individuals aged 65-80y	Vit E	50mg or 100mg	6m	↑DTH (Multitest) in 100mg group (near-significant) ↑IL-2 (non-significant)
Fortes[269]	118 individuals living in a home (mostly self-caring), aged ≥65y	3 intervention groups: 1) Vit A; 2) Zinc; 3) VitA+Zinc	Vit A: 800μg Zinc sulphate:25mg	3m	Zn groups[a]: ↑%CD4$^+$ cells, ↑T_c cells Vit A groups[b]: ↓%CD4$^+$ cells, ↓CD3$^+$ cells All groups: no difference in LP
Penn[259]	28 elderly long-stay patients with strokes (mean ages 83.5y & 83.9y)	Vit A+Vit C+Vit E	Vit A: 8000iu; Vit C: 100mg Vit E: 50mg	28d	↑T cells; ↑%CD4$^+$ cells, ↑%CD4$^+$:CD8$^+$ ↑LP } Unclear if significantly > placebo
Bogden[270]	56 independently living healthy individuals aged 59-85y	Multivitamins/minerals (22 micronutrients)	20-450% RDA	12m	↑DTH (Multitest)
Boardley[271]	31 nuns living in residential home aged 65-89y	Multivitamins/minerals (22 micronutrients)	20-450% RDA	10w	No difference in CD3$^+$, CD4^4, CD8$^+$ or NK cells No difference in LP
Chandra[272]	86 independently living elderly individuals aged >65y	Multivitamins/minerals (18 micronutrients)	USA RDA, except β-carotene & Vit E (=4x upper quartile)	12m	↑T cells, ↑%CD4$^+$ cells, ↑LP ↑IL-2, ↑NK cells; no difference in CD8$^+$ cells
Pike[269]	35 healthy independently living individuals aged 61-79y	Multivitamins/minerals (16 micronutrients)	Vit A: 800RE; Vit B_6: 3.65mg; Vit C: 90mg; Vit E: 45mg; Folic acid: 0.4mg; Iron: 27mg; Zinc: 22.5mg	12m	↑T cells, ↑CD8$^+$ cells ↑NK cells No difference in LP at 12m; ↓ LP at 9m

↑=significant increase in supplemented vs placebo ↓=significant decrease or significantly less change in supplement vs placebo; ConA = concanavalinA; PHA = phytohaemagglutinin; SAC=*Staph. aureus* Cowan1
CD4$^+$ = helper T-cells; CD8$^+$ = cytotoxic / suppressor T-cells; DTH = delayed type hypersensitivity; NK=natural killer (cells); LP=lymphocyte proliferation; RDA = recommended daily allowance
[a] Zn/Zn+VitA compared to VitA/placebo [b]VitA/VitA+Zn compared to Zn/placebo [c] Unclear whether the same individuals as in[263]

vitamin C supplementation trial reported significantly increased DTH responses and lymphocyte function in the supplemented group compared to baseline function, but it was unclear whether this increase was significantly greater than the responses amongst controls.[265] The four studies of vitamin E supplementation (used singly or in combination with vitamins A and C) reported significantly increased DTH responses amongst the supplemented group (in three studies), increased T cell subsets (in one study) and increased lymphocyte proliferation to one mitogen (in one study).[259,266-268] Of the three trials of zinc supplementation, one found that T cell subsets were increased in the zinc group, but that lymphocyte proliferation was not significantly enhanced.[269] This study additionally randomised some individuals to vitamin A, and found that these individuals experienced a reduction in T cell subsets. The other two zinc studies supplemented all individuals with a multivitamin/mineral tablet, and reported that DTH was either not significantly increased or (for individuals receiving high doses of zinc) was significantly less than in the placebo group.[263,264] Consideration needs to be given to the effect of overnutrition with micronutrients, as excess intakes of zinc, vitamin E and iron may all have an adverse effect on cell-mediated immunity.[273-275]

Only one of the trials summarised in **Table 2.3** investigated whether micronutrient supplementation reduced susceptibility to infection. Chandra *et al* found that elderly individuals receiving multivitamin/mineral supplements were significantly less likely to have episodes of (physician/laboratory diagnosed) infectious illness during the study compared to individuals receiving placebo (a mean of 23 vs. 48 days per year).[272] Other randomised controlled trials of micronutrient supplementation in the elderly have been designed primarily to investigate infections as an endpoint. Giridon *et al* randomised 81 elderly individuals living in a geriatric centre to daily doses of either 1) vitamins (β-carotene: 6mg; vitamin C: 120mg, vitamin E: 15mg); 2) minerals (zinc: 20mg; selenium: 100μg); 3) both vitamins and minerals, or 4) placebo.[276] After two years, individuals who received 2) or 3) (minerals or minerals with vitamins) had significantly fewer urogenital or respiratory infections compared to the placebo group, but this was not found for those receiving vitamins alone. In contrast, Chavance *et al* found no significant difference in the incidence of self-reported infections amongst 218 healthy independently living elderly individuals given daily multivitamin/mineral supplements (containing 21 nutrients) for four months.[277] Similarly, Murphy *et al* reported the same number of antibiotic-treated infections amongst two groups of nursing-home residents given a single dose of vitamin A, either 200,000iu (n=53) or 1000iu (n=56).[278] Differences in findings may again have been due to the populations studied, dosage and type of micronutrients administered, duration of follow-up and outcome definition and measurement. No study has examined the effect of micronutrient intake on risk of zoster.

2.4 CONCLUSIONS

There is limited information on risk factors for zoster amongst individuals without underlying immunosuppression, other than the increased risk associated with ageing. In addition, little is known about the determinants of the diminution of cell-mediated immunity with age. In this Chapter, existing data have been summarised, and two putative determinants for immunosenescence (and therefore for zoster) have been suggested – UVR exposure and micronutrient intake. The rest of this document reports on the community-based study that was undertaken to investigate risk factors for zoster in individuals without underlying immunosuppression. In the next Chapter, the main objectives and the methods of the study are described.

3. RESEARCH METHODS

3.1 STUDY OBJECTIVES

The primary objectives of the study were to:

1. Investigate whether exogenous exposure to cases of varicella or zoster in the last ten years protects against development of zoster.
2. Evaluate the interrelationship between the effects of ethnicity, country of residence in childhood and age at varicella on risk of zoster.
3. Estimate the effect of micronutrient intake (specifically intake of vitamins A, C, E and B_6 and of folic acid, iron and zinc) in the last year on the risk of developing zoster.
4. Estimate the role of exposure to ultraviolet radiation (UVR) in childhood and in the last year on the risk of zoster.

The secondary objectives of the study were to:

1. Evaluate the effect of psychological stress in the last year on the risk of zoster
2. Investigate whether recent mechanical trauma affects the risk of zoster.

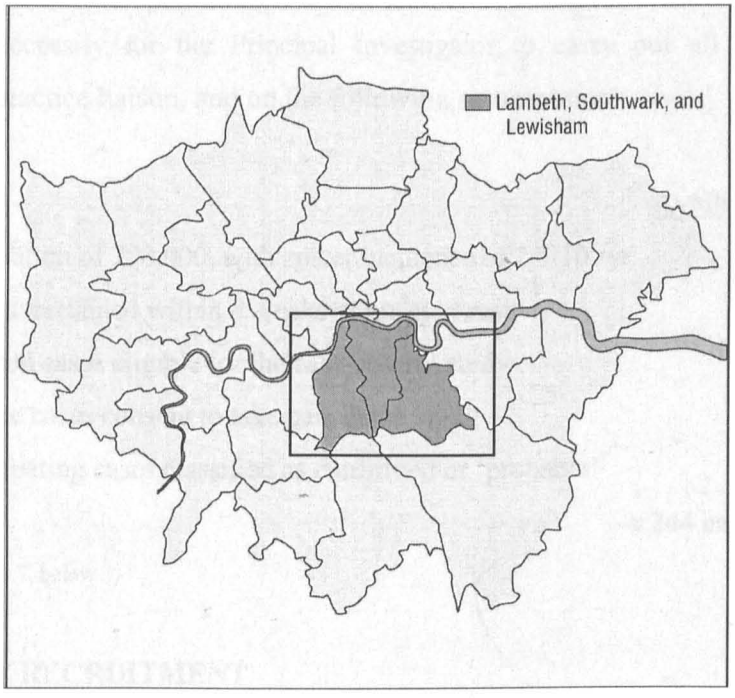
3.2 STUDY DESIGN

The study was conducted in two stages. Firstly, a surveillance system was set up to identify incident cases of zoster in the community. Secondly, a matched case-control study was designed to determine any association between risk of zoster and the exposures listed above. This design offered an efficient approach to examining the numerous exposures of interest. A hospital-based case-control study may have been logistically simpler to carry out, but in the United Kingdom individuals with zoster referred to hospitals are highly selected (mostly those with disseminated rashes or ophthalmic involvement). Therefore, exposures in these cases might not be representative of all cases in the community, and it may have been difficult to identify an appropriate control group. A cohort study design could also have been chosen, but this would have been considerably more time-consuming and expensive, given that the incidence of zoster is typically 2-4/1000 person-years.

3.3 STUDY SETTING

The study was based in South London in the boroughs of Lambeth, Lewisham and Southwark, an area of approximately 35 square miles (**Figure 3.1**). The three boroughs comprise 76 mostly inner-city wards, with an estimated total resident population in mid-1998 of 745,300, of which 63% were aged 16-59 years of age, and 15% were aged 60 years or older.[279] The three boroughs are relatively deprived – Southwark is ranked 9th most deprived district in England, Lambeth 21st and Lewisham 30th (out of 364 districts), using the Overall Index of Multiple Deprivation 2000.[280] However, there is heterogeneity within boroughs. For example, 22 of the 25 wards in Southwark are included amongst the top 25% most deprived wards in England, but one ward is only the 6181st most deprived ward out of 8414 English wards. The boroughs are ethnically diverse, with 26% of residents of non-white ethnicity.[281] The choice of study area therefore facilitated investigation of the effects of ethnicity and country of birth on the risk of zoster.

Figure 3.1: The study setting within Greater London.



3.4 SAMPLE SIZE CALCULATION

Sample size calculations were derived from standard equations for matched case control studies, adjusted for choosing two controls per case.[282] Taking an odds ratio of 2.0, $\alpha = 0.05$ (2-sided), and $\beta = 0.2$, the number of cases and controls needed were calculated for a range of

prevalences of exposure in the control group (p_0):

a) $p_0 = 10\%$: 203 cases and 406 controls. (+20%)[1] → 244 cases and 488 controls

b) $p_0 = 15\%$: 149 cases and 298 controls (+20%)[1] → 179 cases and 358 controls

c) $p_0 = 20\%$: 125 cases and 250 controls (+20%)[1] → 150 cases and 300 controls

[1] Increase in sample size needed to accommodate multivariable analyses

These calculations assume p to be a binary exposure. However, analyses were planned using quantiles of exposure for some of the exposures of interest. The slight increase in sample size needed to accommodate quantiles is offset by the power gained from using statistical tests-for-trend rather than tests for heterogeneity, and by basing quantiles on the distribution of these exposures in controls (discussed in Section 3.10.3, below). Taking all the above into consideration, it was decided that a sample size of 244 cases and 488 controls would be sought.

It was estimated that a combined practice population of 200,000 registered patients would be needed to recruit sufficient cases and controls within 18 months. This calculation was based on the necessity for the Principal Investigator to carry out all the recruitment, interviews and practice liaison, and on the following assumptions

	Available cases/year (n)
1. Practice population of 200,000, with zoster incidence of $2.5/10^3$/yr	500
2. 60% of cases ascertained within 8 weeks of onset of rash	300
3. 80% of reported cases eligible for the case-control study	240
4. 85% of eligible cases consent to take part in the study	204
5. 80% of participating cases classified as confirmed or 'probable'*	**163 cases/yr**
	→ 244 cases in 18 months

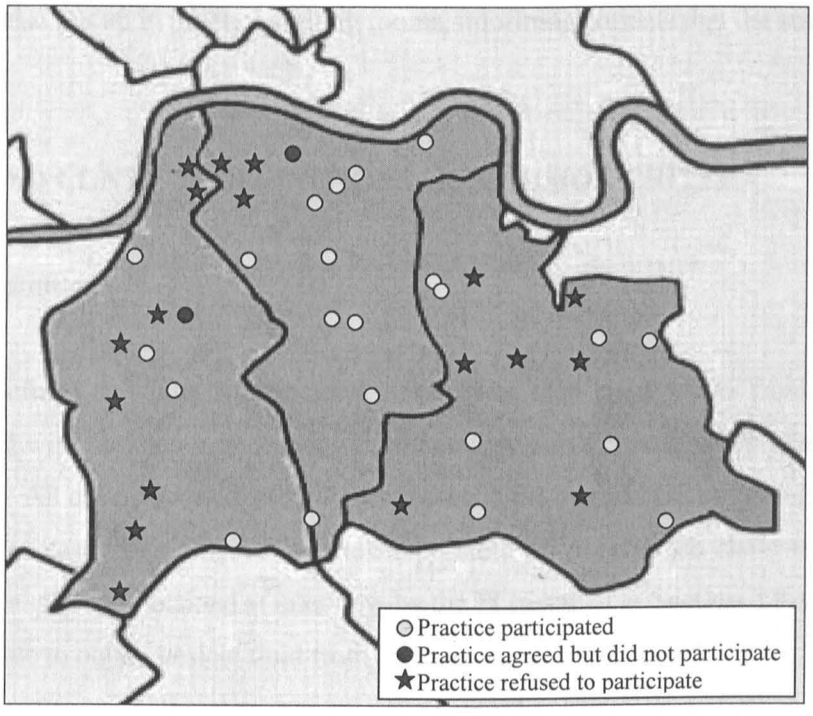
* Defined in Section 3.7, below

3.5 PRACTICE RECRUITMENT

A list of general practices in the three boroughs was obtained from the Family Health Services Authority. All 42 practices comprising three or more general practitioners (GPs) were sent a letter in June 1997, outlining the study and asking them if they would like to participate. The Principal Investigator (PI) followed up the letters by telephoning the senior partner of each practice to ascertain interest, and then visited each interested practice to discuss the study further with practice staff.

Twenty-two of the 42 general practices agreed to take part, as did two smaller practices (each comprising two GPs) that were linked to one of the larger practices. Of these 24 practices, 22 were recruited into the study - one large practice was not enrolled because they appeared insufficiently organised to report cases consistently, and the other practice agreed to participate after a sufficient number of practices were enrolled. Participating practices comprised a total practice population of 198,997 permanently registered patients, with 15.8% aged 60 years or older. The sites of participating and non-participating practices are indicated in **Figure 3.2.**

Figure 3.2: Sites of participating and non-participating practices within Lambeth, Southwark and Lewisham



3.6 CASE SURVEILLANCE

Twenty of the 22 participating practices actively reported incident cases of zoster as they arose to the PI, using a dedicated telephone/fax number. Standardised reporting forms were used. These included information on the date of consultation, the reporting GP, and the patient's name, sex, date of birth and contact details (see **Appendix 1**). Two practices reported cases weekly, after consulting fully computerised consultation records. Hand-searching of paper records of deputising service billings was also carried out in two practices. A study liaison person (usually the practice manager) was identified for each practice, and the PI maintained regular telephone contact with him/her to discuss the practice's ongoing contribution to the study and to sort out any problems. The study liaison person regularly reminded practice staff

about the need to report zoster cases. Additional reminders included the following:

1. Study posters and computer stickers were placed and maintained in all consulting rooms as visual reminders to GPs and practice nurses (see **Appendix 2**);
2. The PI visited all practices regularly;
3. The PI wrote a monthly study newsletter about the study's progress, and sent multiple copies to each practice (see **Appendix 3**);
4. Flyers about the study were provided for locum information packs.

Posters were also put up in practice waiting rooms, informing patients that the study was taking place.

3.7 CASE AND CONTROL DEFINITIONS / EXCLUSION CRITERA

3.7.1 Case definition

Cases were defined as adults with incident zoster (less than eight weeks from rash onset at interview) and with no known underlying immunosuppressive conditions (outlined in **Section 3.7.3**, below). All cases reported to the PI were diagnosed by their GP as having zoster. Viral confirmation of cases was obtained wherever possible by polymerase chain reaction (PCR) analyses of vesicle fluid obtained at interview by the PI (detailed in **Section 3.9.3**) However, it was not possible to obtain vesicle fluid from all cases at interview, due to delays in reporting or interviewing cases or to the presence of maculopapular rashes in a few cases. In these situations, standardised clinical criteria were used to assign unconfirmed cases to one of three categories:

1. *Probable cases* had a unilateral vesicular or maculopapular rash with a dermatomal distribution where a) rash covered more than one quarter of the dermatome, or b) rash was less extensive but pain or dysthesia covered more than one quarter of the dermatome, or c) rash and pain were less extensive, but pain lasted at least one month after rash onset. Individuals with a history of a similar dermatomal rash at any site within the last ten years were excluded from the 'probable' category
2. *Possible cases* had a unilateral vesicular or maculopapular rash with a dermatomal distribution where the rash covered one quarter or less of the dermatome, with either no pain or with localised pain lasting less than one month.

3. *Unlikely cases* either had a rash with a non-dermatomal distribution, or had a history of a similar dermatomal rash at any site in the last ten years.

Individuals without vesicle samples who had 'unlikely' zoster presentations were excluded by the PI at interview. Classification of other clinical cases into 'probable' or 'possible' was carried out by the PI at the end of the fieldwork, without referring to individuals' exposure histories.

3.7.2 Control definition

Controls were defined as adults with no history of zoster who were registered with the same practice that notified the case. Use of self-reporting of a lack of zoster is considered reliable, because the distinctive rash and pain of zoster are memorable. Schmader *et al* investigated accuracy of self-report of zoster among elderly individuals living independently in the community, by comparing responses with physician records and a zoster verification questionnaire.[283] Compared to physician reports, none of the 63 individuals who denied previous zoster were false negatives, and one of 31 individuals (3.2%) reporting a previous history of zoster were false positive. Using the verification questionnaire as the gold standard, the sensitivity of self-report was 95% (40/42) and the specificity was 98% (80/82).

Two controls were selected for each case, individually matched to the case on age, sex and general practice. Age is a known risk factor and sex a potential risk factor for zoster, and both variables were likely to confound many of the associations of interest in this study. Matching by practice may have diminished socioeconomic differences between cases and controls, and reduced the effect of ascertainment bias.

3.7.3 Exclusion criteria

Exclusion criteria for both cases and controls included:

1. Individuals under the age of 16 years
2. Individuals with underlying suppression of cell-mediated immunity. These included a) individuals with HIV infection, b) malignancies considered active in the previous five years, c) autoimmune and other disorders known to be associated with altered cell-mediated immunity (for example, systemic lupus erythematosus or sarcoidosis), and d) immunosuppressive therapies administered in the last six months (such as oral steroids,

cytotoxic drugs used to treat malignancies and cytotoxic immunosuppressants given to transplant recipients or to individuals with autoimmune disorders).

3. Individuals of sub-Saharan African ethnicity. Africans comprise approximately 6% of the population in the study area, and are at higher risk of undiagnosed HIV infection compared to other residents.[281,284] As discussed in the Chapter 2, zoster has an estimated 85-95% positive predictive value for HIV infection in certain sub-Saharan African populations. Therefore Africans with zoster may have been more likely to have undiagnosed or undisclosed HIV infection.
4. Individuals unable to answer questions at interview due to physical or mental impairment, without an available proxy interviewee.
5. Individuals temporarily registered with the practice - these individuals were likely to differ from permanently registered patients with respect to the exposures of interest.

Cases were also excluded if:

1. Vesicle samples were negative for VZV on PCR analysis but positive for herpes simplex virus (see Section 3.9.3, below)
2. Cases were categorised as 'unlikely' using clinical criteria, and vesicle samples were not available or were negative for both VZV and HSV
3. Cases were ascertained more than eight weeks after rash onset

Controls were also excluded if they reported a previous history of zoster.

3.8 RECRUITMENT OF CASES AND CONTROLS

3.8.1 Case recruitment

The PI sent each zoster case over the age of 16 years an introductory letter and an information leaflet about the study (see **Appendix 4** and **Appendix 5**). This was followed up by contacting cases by telephone or by visiting them (if no telephone number was available), to see if they were eligible for the study and were willing to participate. The PI agreed with willing and eligible cases a suitable time to visit them at home to carry out the interview.

3.8.2 Control ascertainment and enrolment

After interviewing each case, the PI visited the notifying practice to ascertain matched controls. Twelve individuals of the same sex and with the nearest dates of birth to the case were initially identified by computer searches of the age-sex register, and the two individuals with the closest dates of birth were sent an information leaflet and a letter about the study, signed by their general practitioner or by the PI (see **Appendix 6**). The PI then contacted these controls by telephone and/or in person to ascertain whether they were eligible for the study and willing to participate. If contact was not made at the initial attempt, further contacts were attempted on at least three different occasions at different times of the day or evening (including one weekend visit) over a period of more than a week. Neighbours were also asked whether they knew of the individual's whereabouts, so that subsequent calls could be planned and individuals who were away on holiday could be included in the study. If controls were ineligible or refused to participate, individuals with the next nearest date of birth on the age-sex register were approached. Further attempts were also made at this stage to contact individuals who the PI had not been able to contact earlier. This process continued until two eligible controls were recruited.

3.9 DATA COLLECTION

The fieldwork lasted for sixteen months, from September 1997 to December 1998 inclusive. Cases and controls who agreed to participate were visited by the PI at a pre-arranged time, usually in their own homes. If a participant cancelled or failed to appear at the meeting, at least four further attempts were made to arrange a second meeting.

The visit comprised:

- a fuller discussion about the study
- final checking of eligibility
- obtaining written informed consent for participation
- administration of a standardised questionnaire
- anthropometric measurement
- collection of a vesicle fluid sample from cases for PCR analysis.

3.9.1 The questionnaire

The questionnaire was designed to confirm eligibility for the study, to obtain clinical information about zoster in order to categorise cases without vesicle samples, and to elicit data on the exposures of interest and possible confounders. A copy of the final questionnaire can be found in **Appendix 7**. It was developed after considering the methods and questions used in a number of previous questionnaires, was piloted in August 1997 by interviewing ten individuals living in South London (4 males, 6 females) aged 46-84 years, and amended slightly before use in the main study.

The questions and methods used are summarised below.

General / medical history: questions were asked about date of birth, housing tenure and car access, and recent medical history, treatments and procedures

Details of zoster: all participants were asked about past episodes of zoster, and cases were asked about pain, rash and other symptoms during the current zoster episode.

Residence and job calendars: information was sought on dates of residence for each place of residence in the participant's lifetime. Job history from the age of 14 years was also obtained, with the number of days worked per week. These data formed the basis for later questions about ultraviolet radiation (UVR) exposure and occupational contacts with children or ill individuals (discussed below), and provided information on country of childhood. The calendars were initially sent to cases and controls with the invitation letter, to self-complete before the main interview if they so wished. Within the first three months of the main study, it became clear that participants preferred to complete the calendars at interview, and this was carried out for the remainder of the study.

Ethnicity and history of chickenpox: ethnicity was self-defined. Participants were asked whether they remembered having chickenpox, and if so at what age.

Contacts with cases of varicella or zoster, and occupational/social contacts with children: questions were asked about contacts with cases of varicella or zoster in the last 10 years, and lifetime occupational contacts with ill individuals. However, varicella is infectious before rash onset and some contacts may have been unrecognised.[285] As varicella is mostly acquired before the age of ten years in the United Kingdom,[82] additional data were sought on contacts with children aged 1-10 years as surrogates for exogenous varicella exposures.

Questions were asked about social contacts with children in the last ten years, including 1) specific children living in the household, 2) specific children not living in the household, such as grandchildren and neighbours, or 3) a range of different children in groups with changing membership, such as at school playgrounds or parties. The job calendar was used to identify lifetime occupational contacts with children.

Past UVR exposure: the objectives of this thesis were to examine the effects of childhood and recent UVR exposures on the risk of zoster. Childhood exposure was chosen to examine the hypothesis that high levels of UVR exposure in early life might 'programme' the immune system to respond less robustly to subsequent challenges. However, data were collected for lifetime UVR exposures, for analysis at a later date. What follows is a brief description of the UVR data that were collected for the present analyses.

The job and residence calendars were used to ask about the number of hours spent outdoors between 9am and 5pm in summer and in winter for childhood (age 7-8 years) and in the last year, and these data were then converted into UVR exposure using data on latitude and cloud cover for each geographical location (detailed in Chapter 4). The questions were based on the questionnaire developed by Kricker *et al*, which has been widely used in studies of skin cancer.[286] Questions about past UVR exposure are difficult to validate formally, but lifetime UVR exposure ascertained from the questionnaire has been shown to correlate with degree of benign cutaneous sun damage, and to be predictive for risk of basal cell carcinoma.[287] Kricker demonstrated that use of residence and job calendars maximised participants' recall by relating each question about time outdoors to where they were living and working at that time. In this study, individuals were asked about how long they spent outdoors between 9am-5pm in childhood and in the last year on:

- **work (or school) days:** 'work' was defined as the occupation that took up the major part of the week, and included periods of retirement, full-time childcare, unemployment and (for childhood) school-days. Additional questions were asked to help participants recall outdoor exposure on school-days, including a) whether the participant walked to and from school, and how long this took, b) how long breaks were in the day and for lunch, c) what time school finished, and d) whether the participant went out to play after getting home from school.

- **non-work days:** these comprised the days when not 'working' or going to school. For exposure in childhood, separate questions were asked about non-school days (weekends) and school holidays. A separate question was asked about time spent outdoors sunbathing in adulthood.

- **summer and winter holidays:** information was collected on both time spent outdoors and duration of holidays. For holidays in the last year, the dates of the holiday were sought, in order to obtain a more accurate estimate of recent UVR exposure.

Data were also sought on use of hats and protective clothing on work days, non-work days and holidays, propensity to burn and ability to tan, history of severe sunburns, and non-sunlight UVR exposures (use of sunbeds, and occupational and therapeutic UVR exposures).

Average micronutrient intake in the previous year and in the two months before rash onset was assessed using a food frequency questionnaire (FFQ) and questions about consumption of dietary supplements. Food frequency questionnaires typically comprise two or three components: what foods are eaten, how often they are eaten, and (sometimes) information on the portion size consumed, all over a specified period.[288] These data can then be converted to micronutrient intake, using food composition tables. The advantages of FFQs are that firstly, they are easy to administer and are well accepted by participants. Secondly, they allow estimation of average (as opposed to short-term) intake. Thirdly, they seek information on food intake before disease onset. Alternative methods used to assess micronutrient intake include measurement of serum micronutrient levels, recall of all dietary items eaten in the last 24 hours, and keeping weighed records of all food items consumed for a specified number of days. These methods have disadvantages when used in case-control studies. For example, they all measure intake after disease onset (which may be unrepresentative of previous intake), and single biochemical measurements of some micronutrients and 24-hour recall may correlate poorly with average micronutrient intake because there can be substantial within-person variation in intake.[289,290]

The FFQ used in this study was a modification of Willett's FFQ, which was first developed for use in a cohort of over 10,000 US nurses.[291,292] The original questionnaire comprised a list of foods of nutritional interest that were commonly eaten by US individuals. There were frequency options for each food (ranging from less than once a month to more than six times a day) and a semiquantitative approach was used – questions referred to standard portion sizes such as a slice of bread, a tablespoon of cream or a 'medium serving' of meat. Collection of more detailed individual portion size data may add little useful information because a) individuals often find it difficult to estimate how much they eat of foods that do not come in easily-defined units (leading to increased interview time and possibly decreased compliance),[293,294] b) there may be considerable within-person variation in portion size,[295] and

c) variations in micronutrient intake between individuals of the same sex and similar age may be largely determined by frequency of consumption.[296-298] The questionnaire was validated on a sample of 194 nurses, by comparing micronutrient intakes in the last year estimated from the FFQ with intakes estimated from multiple weighed diet records taken over the same period.[291] Correlations ranged from 0.36 (for vitamin A intake without supplements) to 0.75 (for vitamin C intake with supplements). Subsequent validations against biochemical indicators reported correlation coefficients of 0.30-0.63 for micronutrients.[290]

Willett's questionnaire has been modified for use in a variety of populations, and has been extensively validated.[299-308] A UK version was developed as part of a multi-centre European cohort study of cancer and nutrition.[309] This questionnaire used the same frequency categories as Willett *et al*, but the food list was changed to allow for differences in diet between US and UK populations and to obtain accurate information on milk consumption and types of breakfast cereal and fat used.[304,310] It has been validated on more than one occasion, with correlation coefficients for a variety of micronutrient intakes of 0.43-0.59 compared to estimates from weighed records, coefficients of 0.44-0.45 compared to plasma vitamin C levels, and a coefficient of 0.13 compared to plasma measurement of β-carotene.[304,307,308] A study of the reproducibility of the FFQ for micronutrient intakes reported correlation coefficients in the range of 0.47-0.78 for men and 0.59-0.85 for women.[308] The questionnaire underwent a slight further modification for use in a UK study of micronutrient intake in the elderly, to allow for seasonal fruit and vegetable consumption (Prof. A Fletcher, personal communication).

The present study used the modified UK questionnaire (used in the study of the elderly), which included a standard food list of 139 food items. An open-ended section was added for recording foods not listed on the form, to ensure that foods commonly eaten by different cultural groups were included. For each food item, nine frequency options and semiquantitative portion size information was sought, as with the Willett questionnaire. Changes in the frequency of consumption over the last year for any food and seasonal consumption of specific foods were noted. Questions about dietary supplements included the brand of supplement, dose and frequency of consumption. The PI studied the FFQ training manual that was developed for interviewers in the UK study of the elderly, and discussed the questionnaire with one of the trainers before administering it in the present study.

Alcohol and cigarette consumption in the last year: both these factors may also affect cell-mediated immune function, and so could confound some of the exposures of interest.[311-314]

Questions about units of alcohol consumption were embedded in the food frequency questionnaire. Participants were also asked whether they were current, ex- or non-smokers, and about cigarette consumption in the last year.

Stressful events: questions about major stressful events in the last year were adapted from the questionnaire used by Schmader *et al* in their study of the effect of stressful events on zoster,[153] which was itself developed from the Geriatric Scale of Recent Life Events.[315] As the questionnaire for the present study was lengthy, twelve main questions about stress were used, together with an open question about whether participants had experienced any other stressful event in the last year. All participants were asked about recent bereavements, major illnesses amongst family or friends, divorce or separation, difficulty with family members, moving house, problems with neighbours, serious financial worries, and difficulties at work (for those still working). Questions from the original questionnaire that were omitted included:

- multiple questions about the same topic (for example, seven separate questions about work)
- less common events (such as going to jail) that were likely to be reported in response to the open question by all individuals who experienced them
- events that were least likely to be stressful - for example, an improved financial situation.

Physical trauma: questions were asked about injuries in the last six months. Information about surgical and other invasive procedures was obtained from the medical history (discussed above).

3.9.2 Vesicle fluid samples

Wherever possible, a swab of vesicle fluid was obtained by the PI from each case. The specimen was collected on a sterile cotton-tipped applicator, which was then broken off into a container of sterile saline and sent by post to Dr Judith Breuer (Department of Microbiology, the London Hospital). In some instances where vesicle fluid was not obtainable a crust sample was taken instead. Viral confirmation was carried out by amplifying viral DNA using polymerase chain reaction (PCR). Samples were initially tested using single-round PCR for the *Pst*1 site within gene 38 of the viral genome. Negative samples were retested in three ways, 1) using the Taqman system (which can detect very low copy numbers) with primers amplifying the *Pst*1 site within gene 38,[316] 2) using nested PCR with primers amplifying gene

29 and 3) using nested PCR with primers amplifying gene 63. Samples that remained negative for VZV on all tests were stored, together with PCR-positive samples from cases with atypical clinical features. These samples were later tested for herpes simplex virus (HSV) DNA, using PCR with primers that could detect HSV1 and HSV2 simultaneously.

3.9.3 Anthropometric indices

Single body measurements can be combined to provide information on nutritional status. For example, body mass index (BMI) is a measure of under- or over-nutrition in adults that standardises weight for height (BMI = weight (kg)/height(m)^2). However, difficulties in standing or spinal curvature can complicate height measurement in elderly populations. Alternatives to height such as demispan (the distance of the outstretched arm from the sternal notch to the finger roots) can be used. Indices that standardise weight for demispan comprise demiquet (weight(kg)/demi-span(m)^2) in men, and mindex (weight(kg)/demi-span(m)) in women. All these indices are influenced by food intake, energy expenditure and past or present health.

Three anthropometric measures were taken at interview: 1) height to the nearest 0.1cm, using a Microtoise height measure, 2) weight to the nearest 0.1kg, using Soehnle electronic scales, and 3) two demispan measurements to the nearest 0.1 cm using a metal retractable demispan tape, to obtain a mean demispan value. The PI attended a training session for taking anthropometric measures set up for the UK study of the elderly, before embarking on measurements for the present study.

3.10 DATA MANAGEMENT

Data were manually checked by the PI after interview. They were then coded and double entered into multiple databases. After the data were validated, they were transformed, reduced to individual-level data, cleaned and categorised before data analysis.

3.10.1 Data coding

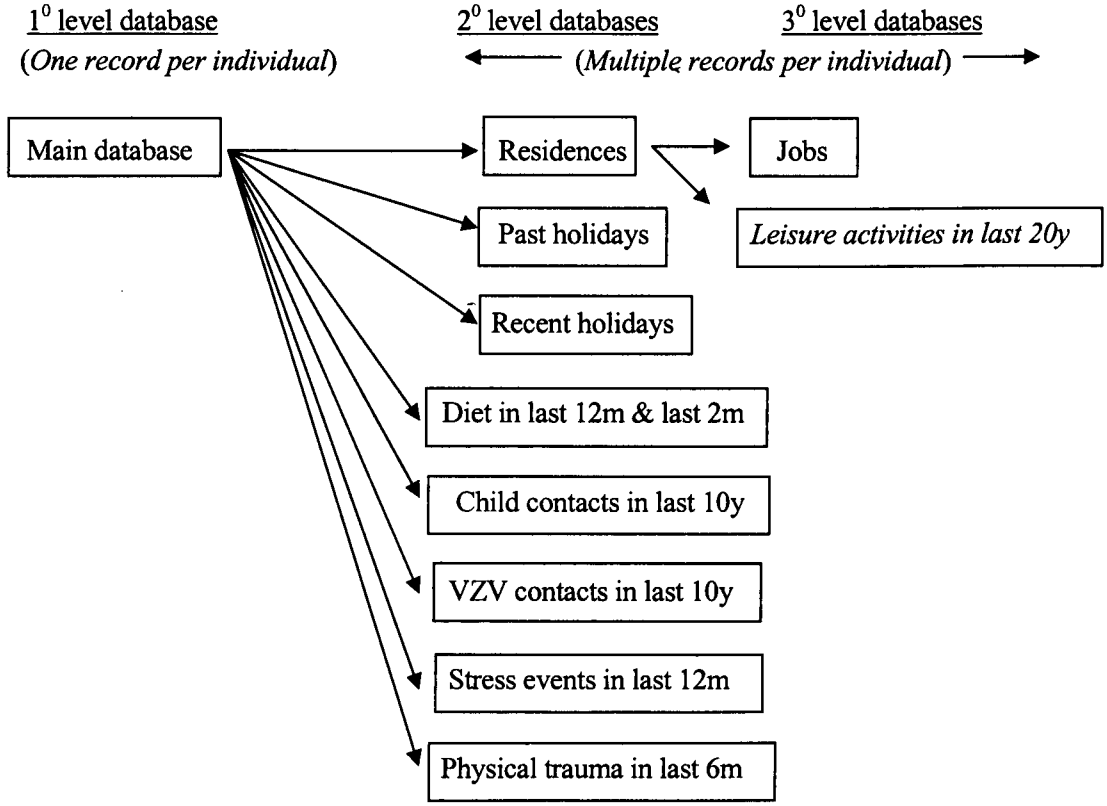
Where possible, variables were pre-coded on the questionnaire. Coding schemes were developed for other variables, and used by the PI to code responses. Data on residences and holiday destinations were coded by latitude, hemisphere and mean percentage cloud cover. Latitude was determined by looking up each place of residence or holiday in a comprehensive world atlas.[317] The mean percentage cloud cover data for warmer and cooler half-years and for

three-month periods were obtained from maps of climatological data.[318] Frequency of food consumption in the two months before rash onset (as opposed to consumption in the last year) was recoded using the seasonal information and other changes in diet noted on the FFQ.

3.10.2 Data entry

Data entry templates were created in Epi-Info 6,[319] using a hierarchical file structure. The main (primary level) database contained a single record for each individual. This held person-level data, such as clinical details of zoster and other current illness, history of sunburn, frequency of holidays, smoking and socioeconomic variables. The main database was linked to ten secondary- or tertiary-level databases, each of which contained multiple records per individual for repeated variables such as child contacts, residences, jobs and holidays. The relationship between the databases is summarised in **Figure 3.3**. The secondary and tertiary databases opened automatically from the main database at certain stages of data entry if there were data on repeated variables for that individual, so that all the data on the questionnaire could be entered sequentially. The PI then double-entered the data, using interactive checking for legal values and ranges, and validated the entries using the EpiInfo Validate programme.

Figure 3.3: Relational database structure used for data entry



3.10.3 Data transformation, reduction, cleaning and categorisation

Datasets were transferred into Stata v.6.0.[320] New variables on the duration, level or number of exposures were created - for example, the level of UVR exposure and the number of child contacts in the last year were calculated from the original data by combining data within and between databases. These new variables were then summed or otherwise summarised to obtain individual-level data. Details of variables that were generated are given in Chapter 6.

Contradictory responses were identified using consistency checks on original and derived variables. The distribution of each exposure variable was examined separately for cases and controls, using histograms and point plots. Outliers were checked against the original questionnaire, and any errors were corrected before data analysis. Programming errors were checked by manually calculating values of derived variables for a sample of data, and comparing the results to those obtained by programming.

Most quantitative variables were categorised into quintiles of exposure, based on the distribution of the variable amongst controls. Where there were large numbers of individuals with no exposure, this was included as a separate category. Using the exposure distribution in controls resulted in increased power to detect significant effects for each exposure level compared to the use of pre-defined cutpoints, and reflected the exposure distribution in the underlying (age- and sex-matched) population. Other variables were grouped by generating categories considered relatively homogenous with respect to risk of zoster, and studying frequencies to ensure that wherever possible there were sufficient numbers of individuals in each category.

3.11 DATA ANALYSIS

Data analyses in this thesis are confined to the datasets of 'confirmed' and 'probable' cases and their matched controls (excluding matched sets with 'possible' cases). This decision was taken in order to exclude as far as possible unconfirmed 'zoster' cases that in reality had HSV infection. Exclusion of HSV cases was particularly important for the analyses of the effect of recent UVR exposure on risk of zoster, because UVR is a known risk factor for HSV reactivation. Therefore, inclusion of HSV cases might result in an overestimate of the effect of UVR on zoster. In addition, inclusion of HSV cases could lead to an underestimate of the effect of exposures that were not associated with risk of HSV infection.

The analysis was carried out in stages. For each group of related variables, descriptive analyses were followed by univariable analyses for the exposures of interest, and then multivariable analyses were undertaken. A final model was created by combining the results from the group-level analyses.

3.11.1 Descriptive analyses

Reporting of zoster cases by individual general practices over time and the numbers of confirmed, probable, possible and unlikely zoster cases ascertained were described. The age and sex distribution of cases was summarised, as were clinical features of zoster including rash, pain, antiviral use and previous zoster episodes.

3.11.2 Univariable analyses

As explained above, the dataset for these analyses was restricted to cases with confirmed or probable zoster, and their matched controls. Odds ratios were estimated for exposure and confounding variables using conditional logistic regression in Stata v.6.0, with zoster as the outcome variable. The baseline variable was usually taken as the lowest quintile of exposure or the group considered at lowest risk of zoster. The statistical significance of associations between exposure variables and risk of zoster was determined using likelihood ratio tests of heterogeneity and of trend, and 95% confidence intervals were determined using Wald tests. Univariable analyses identified variables for initial inclusion in multivariable models, based on their degree of association with zoster (discussed below).

3.11.3 Multivariable analyses

A large number of exposure variables were potentially associated with risk of zoster. Therefore, separate models were initially set up for five groups of risk factors:

1. Child contacts and contacts with cases of varicella or zoster
2. Ethnicity, country of childhood and age at varicella
3. UVR exposures, history of sunburn, and protective behaviours (e.g. wearing a hat)
4. Dietary exposures
5. Stress events and recent illness

For each model, variables associated with zoster on univariable analysis to the level of $p \leq 0.2$ were included. Two main modelling strategies were used. For some models, exposures strongly associated with risk of zoster were added first, followed by exposures more weakly associated with zoster. For other models, a conceptual framework was used to explore the interrelationship of exposures thought to lie at different points on the same causal pathway. In these models, variables were categorised according to their proximity to risk of zoster and were added in turn to the model, starting with distal variables and ending with proximal variables. The modelling strategies for individual models are discussed in more detail in Chapter 6. For all models, variables were kept in the model at each stage if they remained significantly associated with risk of zoster ($p \leq 0.1$), or if they confounded any of the other variables of interest. A variable was considered a confounder if it changed any of the effect estimates of interest by ≥10%,[321] with no marked increase in standard errors (≤ 20%). Variables excluded at earlier stages of the analysis were added again to later models, to assess whether they became significantly associated with zoster in the presence of other variables. Effect modification of exposure variables by age was investigated in each model, to identify possible determinants of immunosenescence. For these analyses, the study population was split into two groups - 'younger' (<60 years) and 'older' (≥60 years) individuals. This cut-off was chosen because it resulted in groups of roughly equal size, and because limited data from published research has shown that individuals aged greater than 60 years have lower levels of cell-mediated immunity compared to younger individuals (Chapter 2, Section 2.3.3.2). A final model was then set up, combining the findings of the five individual models. In this way, the independent effects of all variables on the risk of zoster were estimated.

3.12 ETHICAL ISSUES

Ethical approval for the study was granted by the Ethics Committees of the London School of Hygiene and Tropical Medicine, and by the four local research ethics committees in the area. If cases refused to allow the GP even to give their name to the PI, the practice reported only their date of birth and sex. Written information on the study was provided to all eligible cases and controls with the initial letter, and the PI discussed the study with potential participants and answered their questions. Written consent was obtained before administration of the questionnaire. Identification of controls was based on their proximity to cases in age, their sex and general practice, and medical records were not used by the PI to determine past history of zoster.

Participant confidentiality was ensured by identifying participants by numerical codes. Patients' names and addresses were not used on the questionnaire or on computerised databases, and access to all patient data was restricted to the study investigators.

3.13 SOURCES OF FUNDING

The PI obtained a studentship award from the Medical Research Council, from September 1996 - August 1999. The Research Foundation for Microbial Diseases in Osaka also donated £40,000 towards the research. This provided a salary for the principal investigator for the fourth year of research, and contributed towards outstanding study expenses.

4. TRANSFORMING DATA ON HOURS SPENT OUTDOORS AND FOOD INTAKE

Chapter 3 included a general overview of the transformation of exposure variables. Specific descriptions of derived variables are given in Chapter 6. However, detailed analyses were needed before hours spent outdoors could be transformed into ultraviolet radiation (UVR) levels, and before usual food intake could be transformed into micronutrient intake. This Chapter comprises a summary of these analyses.

4.1 TRANSFORMING UVR DATA

The amount of UVR reaching the ground at any specific location depends on a number of factors, including latitude, season, altitude, time of day, amount of cloud cover and stratospheric ozone levels.[172] Therefore, differences between individuals in personal UVR exposure are due to three main components: 1) differences in ambient UVR levels at their residences and holiday destinations over their lifetime, 2) differences in the amount of time they spent outdoors at each of these locations at various times of the year, and 3) whether they used protective measures against UVR exposure, such as hats or protective clothing.

Data were collected at interview on the time spent outdoors at various residences and holiday destinations, and on the use of protective measures. Before this information could be converted into personal UVR exposure levels, it was necessary to convert the data on geographic locations into seasonal levels of ambient UVR.

4.1.1 Calculating ambient UVR levels for residences and holiday destinations

Direct measurement of UVR levels at the Earth's surface are made with instruments such as broadband meters, scanning spectoradiometers or satellite-based instruments.[322] Networks of recording stations have been set up, but equipment is expensive and requires detailed calibration. An alternative approach is to use a computer model to estimate ambient UVR levels at specific locations at different times of the year and day.[323] One of the best known models was developed by Green *et al*, who used data on latitude, ozone layer thickness, season and time of day to calculate UVR levels in the Northern Hemisphere.[324-326] This model has been adapted by a number of other researchers, and has been validated by comparing UVR values derived from the model with direct measurements of UVR at the Earth's surface.[323,327] Diffey and Elwood expanded the model to produce tables of ambient UVR levels (expressed in MED) under clear sky conditions.[328] The table gives UVR levels for each month at every 10^0 latitude from 60^0N to 60^0S, from sunrise until different times of the day.

For this study, the level of UVR from 9am to 5am at each 10^0 latitude were obtained from Diffey and Elwood's tables, for warmer and cooler half years and for each month of the year. Northern and Southern hemisphere data were modelled separately to estimate UVR levels for intermediate latitudes in each hemisphere. Quadratic, cubic or quartic equations were fitted in turn to the data, and likelihood ratio tests were used to determine which equation gave the best fit. An example is given in **Figure 4.1** (overleaf), where the quartic equation offered the best fit for Northern Hemisphere latitude data for January. The resulting equations were used to estimate UVR exposure between 9am and 5pm for every residence and holiday destination under clear sky conditions at different times of the year.

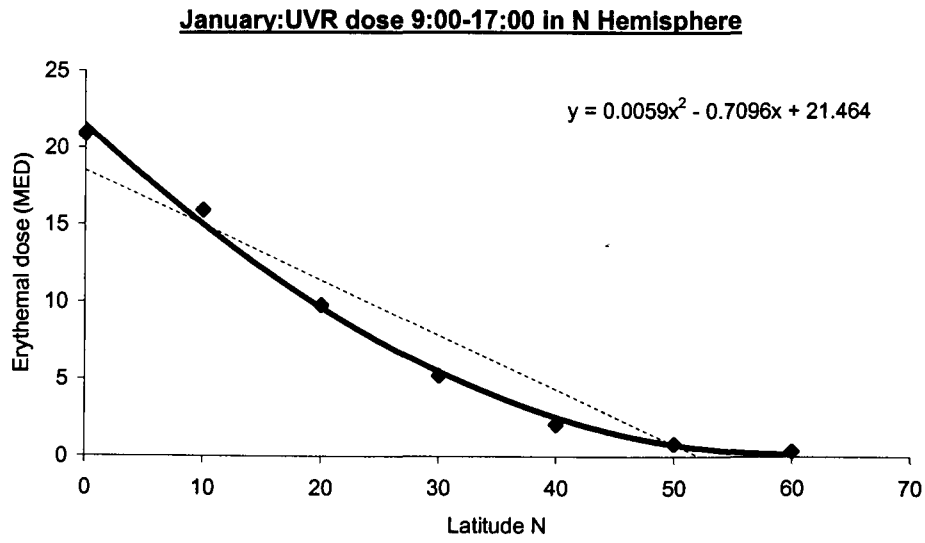
The above estimates took no account of the effect of cloud cover. Seasonal data on mean percentage cloud cover for each location were obtained from maps of climatological data.[318] The level of ambient UVR in each location was adjusted by multiplying clear sky UVR levels by a 'cloudiness factor', calculated as follows:[328]

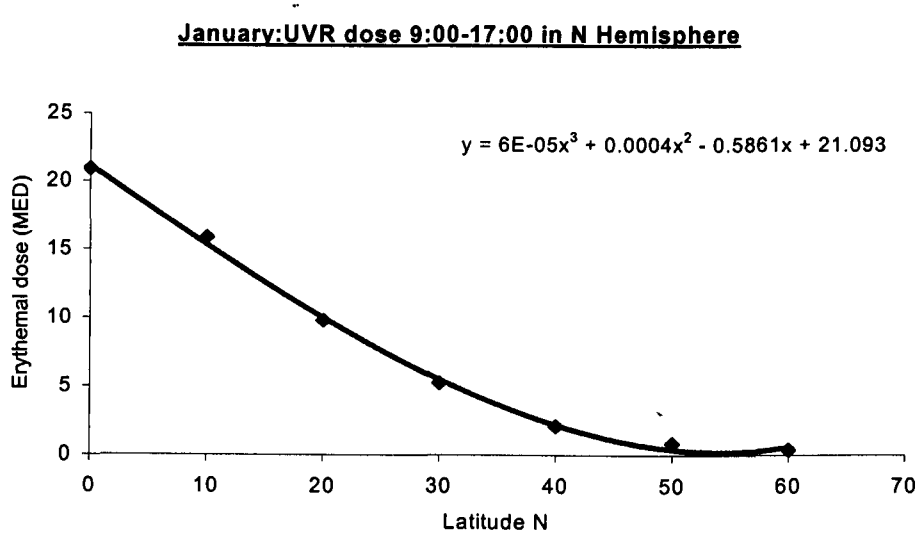
$$\text{Cloudiness factor} = 1 - (0.005 \times C) \qquad (\text{where } C = \text{percentage cloud cover})$$

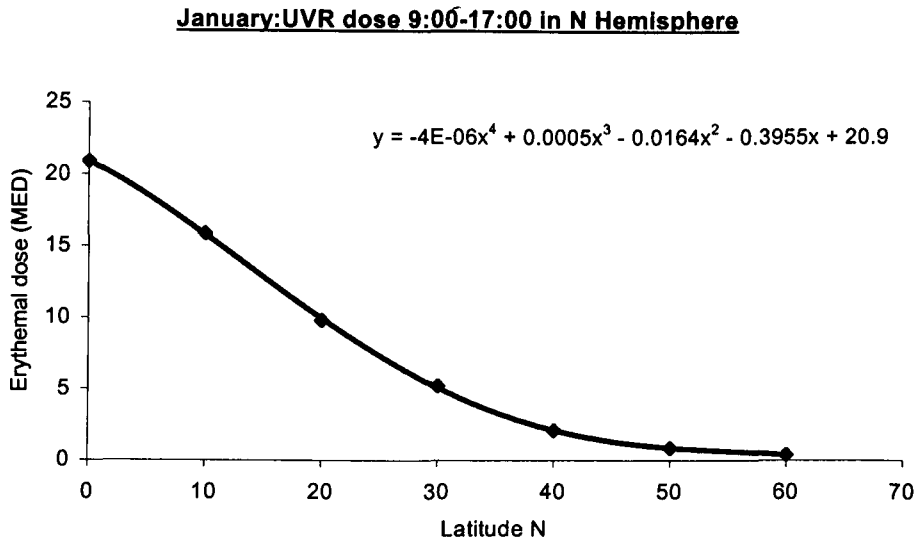
4.1.2 Converting questionnaire data into UVR levels

Data on ambient UVR levels at residence and holiday locations were combined with questionnaire data on time spent outdoors, to obtain personal UVR exposure in childhood and in the last year. Firstly, daily non-holiday UVR exposure was calculated by multiplying the proportion of time spent outdoors between 9am and 5pm on a work (or school) day or a non-work day by the ambient UVR levels for the residence. For example, using the equations described above it was estimated that one hour spent outdoors between 9am and 5pm in the warmer months in London would result in a sub-erythemal UVR dose of 0.84 MED. Secondly, data on daily UVR doses on work- and non-work days were combined to obtain weekly non-holiday UVR exposure, for warmer and for cooler half-years. Similar calculations were carried out to estimate total holiday UVR exposure in childhood. It was possible to obtain a more precise estimate of holiday UVR exposure in the last year, because information was collected at interview on the month in which these holidays were taken. Therefore, recent holiday exposure was calculated using ambient UVR levels for the specific month at the holiday destination. Similarly, non-holiday UVR exposure in the month before rash onset was calculated using ambient UVR levels for that month at the individual's residence.

Figure 4.1: **Fitting of quadratic, cubic or quartic equations to UVR levels by latitude**







After personal levels of UVR exposure had been calculated, data were categorised into quintiles of exposure, based on the exposure distribution amongst controls. No adjustment was made at this stage for the use of protective measures against UVR, such as hat-wearing. These measures were later added as separate variables in the analysis, to assess any protective effect against zoster. The analysis of UVR exposures is described in Chapter 6.

4.2 TRANSFORMING FOOD INTAKE DATA

Three types of information were needed to transform food frequency questionnaire (FFQ) data into micronutrient intake:

1. *The frequency with which various foods were consumed:* this was collected at interview.

2. *The average portion size of these foods:* portion size information was easily obtainable for items that were eaten as 'units', such as slices of bread or pieces of fruit.[329] However, questions were asked about 'medium portions' of other foods. The average size of a medium portion was likely to vary by age and sex, and was not immediately available from standard sources.

3. *The micronutrient content of a standard amount (for example, 100g) of each food:* food composition tables list information on the micronutrient content of FFQ food items. In this study, data were sought from the latest edition and supplements of the standard UK tables, *McCance and Widdowson's The Composition of Foods.*[330-340] Highly characterised FFQ food items such as fresh bananas or semi-skimmed milk were easily identified in McCance and Widdowson (M&W) listings. However, other FFQ items fell into broader categories (such as *beef* or *lamb*), for which there were multiple M&W items. For example, McCance and Widdowson lists 91 food items that could be included under *beef*, specifying types of meat (*mince*, *stewing steak*, *sirloin* etc) and methods of cooking (*fried*, *grilled*, *roasted* etc).[334] It was necessary to identify the M&W food that best represented each of the broadly characterised FFQ items.

Data on the commonest foods eaten within FFQ-defined categories and the age- and sex-specific portion sizes of these foods were identified by analysing two large datasets of the dietary habits of English adults, the National Diet and Nutritional Surveys (NDNS). The objectives of the analysis were:

1. To identify the commonest food eaten within each broadly characterised FFQ food, for men and for women of defined age groups and ethnicities

2. To obtain the median portion size for each food item identified from (1) above, by age, sex and ethnicity

4.2.1 The National Diet and Nutritional Surveys

The NDNS Programme has carried out two dietary surveys of adults, 1) a survey of 2197 individuals aged 16-64 years in 1986-87,[341] and 2) a survey of 1687 individuals aged 65 years or older in 1994-95.[342] In both surveys, participants weighed and recorded all foods and beverages consumed in 1) a seven-day or 2) a four-day period. Approximately 5000 and 3000 highly characterised food items were recorded, and these were later coded using an NDNS coding system. The primary data are available from the Data Archive of Essex University.[343,344]

4.2.2 Preliminary data management of NDNS datasets

Demographic and food consumption data were merged to obtain 630,213 coded and weighed food items consumed by 3884 individuals of known age, sex and ethnicity. Forty-two datasets were then created by dividing participants by sex, age (16-24 years, 25-34 years, 35-49 years, 50-64 years, 65-74 years, 75-84 years and 85+ years), and ethnicity (defined by NDNS interviewers as 'White', 'Asian' or 'Coloured').

Some individuals had multiple records of the same food item, because they had eaten this food on two or more occasions during the survey period. This led to two potential problems:

1. Some NDNS foods could be identified as the most common foods eaten within specific FFQ categories not because they were eaten by a wide range of individuals, but because a few individuals ate these foods repeatedly.

2. Average portion sizes for foods eaten on multiple occasions in the NDNS survey were affected by both within-person and between-person variation. These had different determinants - within-person variation in portion size partially arose from eating second helpings and leftovers of the same foods, whereas between-person variation arose mainly from differences amongst individuals in habitual portion sizes.

To minimise these problems, mean portion sizes of foods were calculated for each individual. This ensured that specific foods were only counted once per person.

4.2.3 Identifying the commonest foods eaten for each FFQ category

Every food eaten by an NDNS participant was assigned to one of the present study's FFQ food categories, so that each FFQ food comprised a set of NDNS foods (with up to 175 NDNS foods per FFQ category). If the NDNS food was a composite dish not listed on the FFQ, the component foods were assigned to different FFQ food categories, reflecting the original data collection. For example, the NDNS food *bolognaise sauce* might be assigned separately to the FFQ foods *beef, onions, tinned tomatoes* etc. The commonest NDNS food within each FFQ food category was then identified for each age/sex/ethnicity group. However, individual NDNS foods were very finely categorised and mostly occurred with low frequency within FFQ categories. Therefore, the commonest NDNS food was identified using a two-stage process:

1. The commonest **subtype** of food was first identified - for example, in some age/sex groups varieties of *minced beef* were more commonly consumed than varieties of *roast beef* or *stewed beef.*

2. The commonest **variety** of that subtype was identified – in some groups, the commonest variety of *minced beef* consumed was '*minced beef, stewed, fat skimmed*'.

In some FFQ food categories, all NDNS foods remained at low frequency even after grouping foods into subtypes. In these cases, the commonest NDNS food was identified by combining datasets of individuals of the same sex and adjoining age groups. Limited NDNS data on foods eaten by individuals of non-White ethnicity were supplemented by information collected during interview.

4.2.4 Compiling a nutrient databank

The next stage in the analysis was to set up a nutrient databank, containing information on the micronutrient and energy content of 100g of each food identified for an FFQ category. For most foods, this was achieved by identifying the food in McCance and Widdowson databases accessed using Integrated Dietary Analysis (IDA) Software,[345] and exporting the food composition data. For a minority of foods, there was no M&W listing. In these cases, data on the nutrient content were obtained from the following sources:

1. *NDNS nutrient databank:* this was originally compiled for the NDNS survey of individuals aged 65 years and above.[342] If the food was listed in the NDNS databank, food composition data were exported to the study nutrient databank.

2. *Manufacturer's data:* if foods were not listed in either the NDNS nutrient databank or the M&W listings, food composition data were obtained directly from the manufacturer or from information printed on the packaging. This information was then added manually to the nutrient database.

Data were also collected in the FFQ on the consumption of 222 different vitamin and mineral supplements. The micronutrient content per dose of these supplements was obtained from manufacturers and added to the database.

4.2.5 Assigning portion sizes to questionnaire data

Average portion sizes were then sought for each identified food item. Median portion sizes (in grams) were determined for the foods by sex, age group and ethnicity, using the NDNS datasets. For FFQ items that were eaten as units (such as slices of bread), standard portion sizes were obtained using the IDA Software, which includes a database of Ministry of Agriculture, Fisheries and Food portion size data.[329]

If the NDNS food was an ingredient of a composite dish, the portion size was calculated by multiplying the median portion size of the composite dish by the proportion of the total weight made up by that ingredient. The latter value was obtained from standard NDNS or M&W recipes.

4.2.6 Conversion of questionnaire data to micronutrient levels

Once micronutrient content and portion size had been determined for each food, the food records were converted into daily micronutrient intake for each individual. This was carried out in three stages:

1. The frequency of consumption of each food was converted to daily frequency. Where responses encompassed a range of frequencies, the middle value was taken – for example, a frequency of 2-4 times a week was recoded to 0.429 (3/7) times a day. Frequencies of less than once a month were recoded as 'none'. Frequencies of more than six times a day were recoded as seven times a day, after scrutinising information obtained at interview.

2. Daily micronutrient intakes of each food record were calculated as daily frequency multiplied firstly by micronutrient content per 100g, and then by portion size (expressed as a proportion of 100g). Similar calculations were used to obtain the daily

micronutrient content per dose of vitamin and mineral supplements.

3. The micronutrient intakes from individual food records were summed to obtain daily intakes for each individual of total energy intake (in kilojoules) and the seven micronutrients of interest.

4.2.7 Adjusting for total energy intake

The analysis described above provided estimates of the absolute daily intakes of the seven micronutrients. However, before these could be used in further analyses, the effect of variation in total energy intake had to be removed. Total energy intake varies between individuals due to differences in three main factors:[346]

1. *Body size:* this determines the amount of energy needed for resting metabolic activity;
2. *Physical activity:* there is a strong positive relationship between physical activity and total energy intake. This is probably the major determinant of between-person variation in energy intake.
3. *Metabolic efficiency:* metabolically efficient individuals need less energy to maintain their body size and physical activities compared to metabolically inefficient individuals.

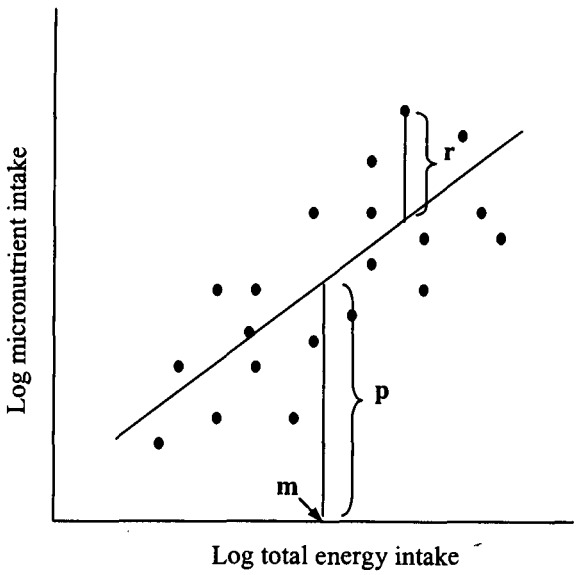
Almost all nutrients tend to be positively correlated with energy intake; in this study, correlation coefficients ranged from 0.262 (retinol equivalents) to 0.817 (zinc) amongst controls for food intake in the last year. It follows that individuals with high energy intake had high absolute intakes of micronutrients. However, most nutrients are metabolised roughly in proportion to total energy intake - larger, physically active and metabolically inefficient individuals tend to have a higher micronutrient requirement compared to small, less active individuals.[346] Therefore, it was necessary to separate the variation in micronutrient intake resulting from differences in dietary composition from the variation due to differences in body size, physical activity and metabolic efficiency. Information on body size is easily obtainable, but data on physical activity and metabolic efficiency are more difficult to measure. As an alternative, total energy intake was used as a proxy for the three variables.

Adjustment for total energy intake was made using Willett's residual model.[346] Firstly, data for micronutrient intake and total energy intake were log-transformed, as they were positively skewed. A simple linear regression model was then set up for each micronutrient amongst controls, with absolute (log-transformed) micronutrient intake as the outcome variable and

(log-transformed) total energy intake as the explanatory variable. The residuals from the regression model were calculated for all participants (**Figure 4.2**). These residuals represented individual variation in micronutrient intake due to dietary composition, separated from the variation due to differences in total energy intake.

Individuals were categorised into quintiles of exposure for each energy-adjusted micronutrient, according to the distribution of each variable amongst controls. The values of residuals provided little sense of the nutrient intake of the diet. The data were transformed into less abstract nutrient levels by adding a constant - the (log) predicted micronutrient value for the mean energy intake amongst controls (see **Figure 4.2**). This was added to each residual value, and then the antilog was taken.

Further analyses of micronutrient data are described in Chapter 6.



r = residual for an individual
m = mean energy intake
p = predicted log micronutrient value for an individual with mean energy intake

Figure 4.2: Energy-adjusted micronutrient intake
(adapted from Willett)[346]

5. DESCRIPTIVE RESULTS

5.1 CASE REPORTING

A total of 436 cases were ascertained between 1 September 1997 and 31 October 1998. Reporting by the 22 general practices over time is summarised in **Table 5.1** (overleaf). The highest numbers of cases were ascertained from the two fully computerised practices (practice nos. 13 and 17) that carried out weekly computer searches for zoster diagnoses. There was no clear pattern in the number of cases reported over time – lowest numbers were reported in the first month of the study, and in January and August 1998.

Of the 436 cases, 421 were actively reported by practices a median two days (range: 0-162 days) after the day of consultation, with 351 (83%) cases reported within a week of consultation. Fifteen other cases were ascertained a median of 64 days (range: 21-139) after consultation, by searching partially computerised practice records.

5.2 ENROLMENT, CATEGORISATION AND CLINICAL FEATURES OF CASES

Of the 436 cases, 139 were categorised as ineligible based on information given by the GP or ascertained by the PI after contacting patients. Forty-six of these cases were less than 16 years old, 37 were immunosuppressed or had a recent history of cancer, 18 were African, 11 were temporarily registered, 4 were incapable of answering questions and 23 were ascertained more than eight weeks after rash onset. Of the remaining 297 patients, 16 (5.4%) were not enrolled due to refusal (12 patients), or being away from London or repeatedly unavailable for more than eight weeks (4 patients). The eligibility of these cases was not ascertained.

The remaining 281 cases were contacted after a median of one (range: 1-12) attempt. Samples were taken from 104 individuals at interview, of which 94 were VZV-DNA positive. The clinical data and VZV-DNA results were used to categorise individuals as confirmed (92), 'probable' (152), 'possible' (18) or 'unlikely' (19) cases (as defined in the previous Chapter, Section 3.7.1). The distribution of PCR results in each case category is summarised in **Table 5.2** (overleaf) and discussed further below.

Table 5.1: Reporting of zoster cases by practice and by month of consultation

Practice	Practice size[b]	Sept 1997	Oct 1997	Nov 1997	Dec 1997	Jan 1998	Feb 1998	Mar 1998	Apr 1998	May 1998	Jun 1998	Jul 1998	Aug 1998	Sept 1998	Oct 1998	TOTAL
1	8532	-	-	2	1	1	-	3	1	2	4	1	-	-	-	15
2, 8, 11[a]	22337	3	1	2	2	3	1	4	4	1	1	9	1	1	3	36
3	7500	-	6	2[c]	4	-	3	-	2	-	-	-	-	1	-	18
4	7388	-	1	-	1	-	4	-	-	-	1	-	2	2	3	14
5	5656	-	-	-	1[c]	1	1	-	-	1	1	-	1	2	-	8
6	10465	1	-	-	1	1	4	2	4	3	3	1	3	1	2	26
7	9934	2	2	1	1	-	2	1	-	3	1	3	1	-	-	17
9	9510	2[c]	1[c]	1	3[c]	-	1	3	-	-	-	-	-	-	-	11
10	5922	2	1	-	1	1	2	2	1	-	2	6	2	3	2	25
12	15078	2[c]	1	4[c]	2	3	1	2	2	3	3	3	-	3	2[c]	31
13	13891	3	4	6	1	1	1	6	9	5	8	2	2	5	6	59
14	9405	-	4	-	3[c]	1	-	5	4[c]	2	2[c]	4	1	2	3	31
15	9199	-	1	-	-	2	-	-	1	1	2	2	-	2	1	12
16	7350	2	2	2	1	-	1	-	1	4	2	-	-	2	2	19
17	16314	4	3	-	3	3	1	5	3	3	-	5	5	1	5	41
18,19[a]	11899	2[c]	2	4	1	-	-	2	-	-	-	2	2	1	-	16
20	6945	-	2	-	-	-	1	1	-	1	2	-	-	1	-	8
21	11884	-	4	4	-	3	2	-	3	1	2	6	2	4	1	32
22	9788	-	1	1	1	2	3	3	1	-	1	1	1	1	1	17
TOTAL	198,887	23	36	29	27	22	28	39	36	30	35	45	23	32	31	436

[a] Linked practices [b] Number of permanently registered patients on 01.09.97 [c] Includes one or more case ascertained retrospectively

Table 5.2: Distribution of PCR results by zoster case category

PCR RESULTS	CASE CATEGORY			
	Confirmed	Probable	Possible	Unlikely
VZV+ve	92	-	-	2[a]
VZV-ve	-	2	4	4
No sample	-	150	14	13
TOTAL	92	152	18	19

[a] See text

Table 5.3: Distribution of rash and pain in enrolled cases (n=262)

PAIN EXTENT	RASH EXTENT PCR-confirmed cases (n=92)				RASH EXTENT 'Probable' cases (n=152)				RASH EXTENT 'Possible' cases (n=18)			
	≥3/4 dermatome	1/2 dermatome	1/3 dermatome	≤1/4 dermatome	≥3/4 dermatome	1/2 dermatome	1/3 dermatome	≤1/4 dermatome	≥3/4 dermatome	1/2 dermatome	1/3 dermatome	≤1/4 dermatome
≥3/4 dermatome	59	3	3	4	85	13	5	7	-	-	-	-
1/2 dermatome	11	3	-	1	11	14	5	3	-	-	-	-
1/3 dermatome	1	2	1		-	1	2[a]	-	-	-	-	-
≤1/4 dermatome	-	-	-	-	-	-	-	-	-	-	-	13
Itching or dysthesia only	-	-	-	-	4	1	-	-	-	-	-	3
No pain	3	-	-	-	-	1	-	-	-	-	-	2
No information	-	-	-	1	-	-	-	-	-	-	-	-
TOTAL	74	8	4	6	100	30	12	10	-	-	-	18

[a] Both cases had pain lasting > 4 weeks after rash onset

The nineteen 'unlikely' cases were excluded from the study. These comprised 12 individuals with 1-24 previous episodes of similar rash in the last 10 years, two cases with rashes that recurred at the same site over the course of the study, four individuals with bilateral, non-dermatomal rashes, and one patient with a non-dermatomal backache without rash. Of the six samples taken from 'unlikely' cases, four were negative for VZV-DNA (**Table 5.2**). One VZV-DNA positive sample came from a patient with a widely distributed bilateral centripedal rash and with no history of varicella. This patient was diagnosed as having varicella. The second positive sample came from a man with no history of varicella who had a bilateral, highly crusting non-dermatomal rash covering a wide area the face and neck, which occurred three weeks after his grandchildren developed similar rashes. This case was considered highly unlikely to be zoster, and was diagnosed as either varicella or impetigo (with a contaminated laboratory sample). All six 'unlikely' samples were negative for HSV-DNA when tested at the London Hospital. However, one 'unlikely' case with a rash with ophthalmic distribution and a history of thoracic 'zoster' seven years previously had a sample taken by her local hospital that tested positive for HSV-DNA.

The 262 confirmed, probable and possible cases were enrolled in the study. These cases were seen by their GPs a median 3 days (range: -5 to 37 days) days after rash onset, and by the PI a median 9 days (range: 0 to 52 days) days after rash onset. In half (131/262) the patients the rashes occurred on the right side of the body. The commonest dermatomes affected were thoracic (57.2%), followed by lumbar (14.9%), cranial (11.8%), cervical (11.1%) and sacral (4.2%). Two (0.8%) patients had rash at two sites, both with extensive dermatomal rashes in the thoracic region and an additional small patch of rash in the sacral region.

The extent of rash and pain within dermatomes is summarised in **Table 5.3** (see previous page). The degree of rash and pain were similar in confirmed and 'probable' cases. Two of the probable cases had negative VZV-DNA results from scab samples – both cases were women in their eighties, with extensive dermatomal rashes and pain. Most (14/18) of the 'possible' cases had only a small patch of rash with localised or no pain. The four 'possible' cases with negative VZV-DNA results were scab samples, each taken from a small patch of rash.

Eighteen of the enrolled cases (4 confirmed, 11 'probable' and 3 'possible' cases) had a history of previous zoster. Of these, sixteen had a single previous episode 12-74 years previously. Two cases reported two previous episodes – one was a confirmed case with two episodes of 'zoster' six months apart 19 years previously, and the other was a 'probable' case aged 81 years with episodes in childhood and 30 years previously. In 16 of the 18 cases, the previous

episodes were at a different body site to the present episode. Two cases had previous episodes at the same site. One was a 'possible' case with a small patch of rash on his thorax. The other was a 70 year-old woman with 'probable' ophthalmic zoster, whose initial episode 15 years previously had resulted in significant pain and scarring, typical of zoster.

Of the 248 cases with known treatment status, 131 (53%) were given oral antivirals, and 20 (8%) were given topical antivirals.

5.3 DEMOGRAPHIC CHARACTERISTICS OF CONFIRMED / PROBABLE CASES

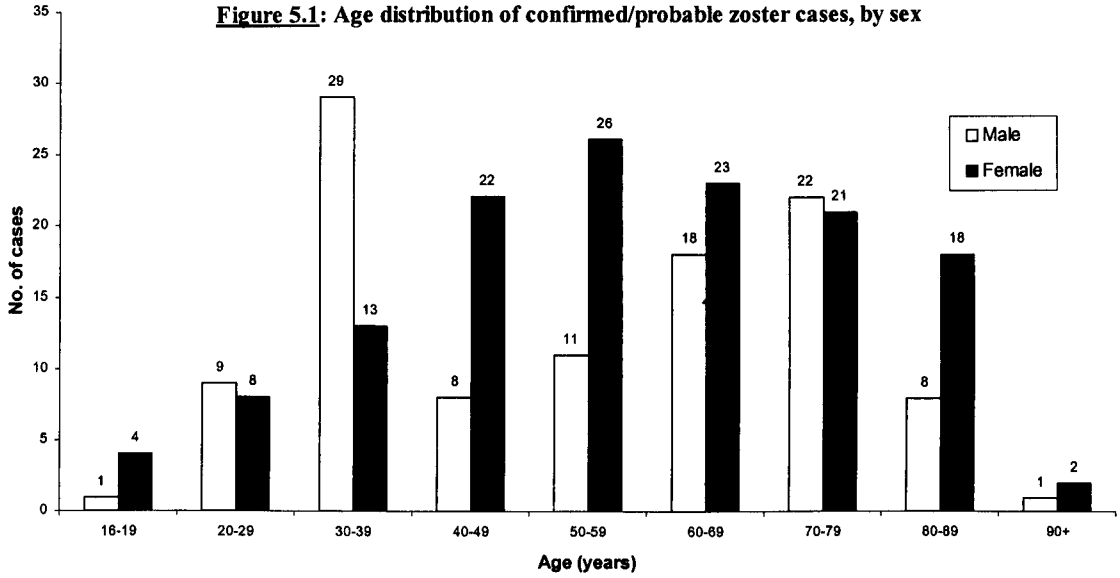
Demographic characteristics were determined for the 244 confirmed and 'probable' cases only, as their data were used in the risk factor analyses. The median age of these cases was 57.2 (range: 16.5-91.2) years. The majority of cases occurred with approximately equal frequency in five age groups encompassing the ages 30-79 years (**Table 5.4**). Overall, 43.5% of cases were male - there was an excess of female cases in most age groups, but a marked excess of male cases (29 males vs. 13 females) aged between 30-39 years (**Figure 5.1**, overleaf).

Table 5.4: Age distribution of confirmed/probable zoster cases

Age group (years)	Registered patients n (%)[a]	Confirmed/probable cases n (%)
16 – 19	N/A	5
20 – 29	33029 (21.6)	17 (7.1)
30 – 39	44467 (29.0)	42 (17.6)
40 – 49	25971 (17.0)	30 (12.6)
50 – 59	18423 (12.0)	37 (15.5)
60 – 69	14164 (9.2)	41 (17.1)
70 – 79	10906 (7.1)	43 (18.0)
80 – 89	5166 (3.4)	26 (10.9)
90+	1120 (0.7)	3 (1.2)

N/A = data not available [a] No / % of permanently registered patients from all practices combined

Ninety percent (220) of the confirmed and probable cases were white, 4% (10) were Afro-Caribbean, 3% (7) were Asian, and 3% were of other ethnicities.



5.4 ENROLMENT OF CONTROLS FOR CONFIRMED AND PROBABLE CASES

A total of 488 matched controls were needed for the 244 confirmed and probable cases. Letters were sent to 895 individuals, of whom 162 were ineligible for the following reasons: 118 had a history of zoster, 22 were immunosuppressed or had a recent history of cancer, 11 were African, one was temporarily registered, and 10 were incapable of answering questions. A further 145 were unsuitable, because they no longer lived in London (106), were living away for extended periods (22), were dead (11) or had an incorrect date of birth on practice records (6). Of the remaining 588 potentially eligible individuals, 103 (17.5%) were not included in the study; 75 (12.8%) refused, 9 (1.5%) twice cancelled interviews, 3 were in hospital, 1 had a non-existent address, and 15 could not be contacted after 4-13 attempts. The remaining 485 controls were enrolled after a median of two (range: 0-21) contacts; only one matched control was obtained for three of the enrolled cases. One control later became a case and was included as both a case and a control. The mean difference in age between cases and their matched controls was 4.7 days.

5.5 DATA COLLECTION FROM CONFIRMED / PROBABLE CASES AND CONTROLS

The 244 confirmed and probable cases and their 485 matched controls were interviewed in their own homes, at their workplaces or elsewhere. Translators were used for seven individuals who did not speak English - six of the translators were family members, and one was a staff member at a Turkish community centre for the elderly. Four proxy interviewees

were used, two of whom were carers for cases with Down's syndrome, one was the wife of a case who was hospitalised, and one was the husband of a control with Alzheimer's disease. A further 91 interviewees (32 cases and 59 controls) were helped in answering questions by a spouse, other family members or (in one case) by a carer. The questionnaire took a median of 67 minutes (range: 30-166 minutes) to administer. One case terminated the interview before completion. Controls were interviewed a median of 35 days (range: 0-141 days) after cases. A total of 22 (4.5%) controls were interviewed more than 90 days after cases. These were mostly the sixth or subsequent control who had been approached for a specific case, where delays were incurred in accessing previous controls who were eventually found to be ineligible, who refused to participate, or who agreed to take part but then failed to attend an interview on two occasions.

Anthropometric measures were obtained for 96% (703/729) of interviewees. Measures were not taken from individuals who could not safely stand on the weighing scales, who refused to be weighed, or who were matched controls of cases with missing anthropometric data.

5.6 DISCUSSION

The target of 244 enrolled cases was attained more quickly than had been estimated, due to a higher than expected number of cases reported by practices, and low refusal rates. Nevertheless, there was underreporting by some practices. This was surmised from the following:

1. Some practices reported fewer cases over time, until no cases were reported (see **Table 5.1**). One practice (practice number 3) reported very few cases after moving premises mid-way through the study.
2. Some cases were not reported when initially seen by one GP, but were reported after a subsequent visit to a second GP in the practice.
3. A high proportion of cases were reported by the two fully computerised practices that performed weekly searches of their computerised records.
4. Searches of partially computerised records identified some cases that had not been reported by the practice.

A recent study of infectious intestinal disease which utilized a GP research framework and dedicated research nurses estimated that practices actively reported only 64% of eligible cases.[347] Reporting levels were significantly lower amongst practices with larger number of

partners and practices with limited research experience. In this study, practice size was not clearly associated with poor reporting (**Table 5.1**). General underreporting of cases by some practices was unlikely to have introduced bias, as controls were matched to cases by practice. However, incomplete reporting might introduce bias if general practitioners were more likely to report cases that fitted the study hypotheses. This was unlikely, as the overall study was investigating a wide variety of risk factors for zoster and these factors were discussed only briefly with GPs at the beginning of the study. In addition, analysis of patterns of practice reporting and of cases that were retrospectively ascertained or reported after second consultations indicated that incomplete reporting was due to overall underreporting by practices or by specific GPs, and not selective reporting of cases.

The common occurrence of rash in thoracic dermatomes has been reported in many community-based studies,[4,17-22,34,64,68] and the distribution of rashes at different body sites in this study was almost identical to that reported in the large Rochester study.[4] The equal spread of cases occurring between the ages of 30 and 79 years reflects an underlying increasing incidence with age, as there were decreasing numbers of patients at risk in the older age groups (**Table 5.4**). Actual incidences were not calculated, as the degree of underreporting in the different age groups was unknown. Similarly, it is difficult to assess any seasonality in the occurrence of zoster – the low numbers of cases reported in August and January probably resulted from underreporting by locums in the months where many GPs were on holiday.

The slight preponderance of female cases has been reported in other community-based zoster studies,[4,22,23,53,62] and in older age groups this is at least partly explained by the higher proportion of females in the population at risk. The excess of male cases aged between 30-39 years is more unusual, although in the Rochester study males aged 35-44 years had significantly higher incidence of zoster compared to females (as discussed in Chapter 2).[4] There are a number of possible reasons for the male excess in this age group. Firstly, males might be more likely than females to visit their GP after they developed zoster. This seems improbable, as the characteristic pain and rash of zoster is such that all individuals are likely to seek medical assistance. Secondly, males of this age may have higher levels of potential risk factors for zoster than females, such as poor nutrition or lack of contact with children. Thirdly, the higher numbers amongst males may reflect undiagnosed or undisclosed HIV infection in homosexual men, a group in London with relatively high prevalence of HIV infection.[284] Inclusion of men with HIV infection may influence estimates of the effect of specific exposures such as contact with children, and this is explored in the analyses of these exposures (Chapter 6).

Samples were not obtained from all cases, due to delays between rash onset and reporting and interviewing cases, rapid healing of vesicles from the widespread use of oral antivirals, and difficulties in carrying out PCR on scabs. The clinical case definition for 'probable' cases was designed to reflect the characteristic presentation of zoster and to exclude cases of herpes simplex virus (HSV) infection. Nevertheless, a small number of atypical HSV cases may have been included amongst probable cases, and a few zoster cases with very limited rash and pain may have been excluded.[36,44] In addition, laboratory error in the diagnosis of confirmed cases cannot be excluded. In the case of recent ultraviolet radiation (UVR) exposure, inclusion of HSV cases may have led to an overestimate of effect, as UVR is a known precipitant of HSV.[348] This is discussed in the section on UVR analyses in Chapter 6. The HSV-DNA negative test results from 'unlikely' cases who had typical clinical HSV infection may have resulted from the use of a single set of HSV-primers with limited sensitivity.

Participation by controls was also high (82.5%). Some bias may have been introduced if the minority who refused to participate or who could not be contacted were eligible for inclusion and had different patterns of the exposures of interest compared to participating controls. A small minority of controls were interviewed more than 90 days after cases. This was not a problem for most exposures, as questions referred to 'usual' exposure in the year before interview. However, controls may have forgotten some specific events that occurred around the time of rash onset in the case, due to the greater time that had elapsed when they were interviewed. This could result in an underestimate of putative protective effects such as contacts with cases of varicella, and an overestimate of risk factors such as stressful events. These and other issues such as potential recall bias are considered in the relevant sections of the next Chapter, and in Chapter 7.

No Page 78 in original

6. ANALYSIS OF EXPOSURES

This Chapter reports on the analyses of the effects of exposures on risk of zoster. It is divided into eight sections. The first five sections describe the analyses for each of the main groups of risk factors – contacts with children and varicella, ethnicity and country of birth, micronutrient intake, ultraviolet radiation exposure, and stress and illness. Each section includes a list of specific hypotheses that were tested, additional data categorisation or modelling strategies that were not detailed in Chapter 3, and results of univariable and multivariable analyses. The sixth and seventh section describe the effects of mechanical trauma and potential confounders on risk of zoster. The final section reports the results of the combined model, which contained selected variables from earlier analyses.

The Tables relating to these analyses can be found on pages 153-193.

6.1 CONTACTS WITH CHILDREN AND WITH CASES OF VARICELLA OR ZOSTER

6.1.1 Specific hypotheses

Two main hypotheses were tested:

1. Contacts in the last ten years with cases of varicella or zoster decreases the risk of zoster
2. Contacts in the last ten years with children aged 1-10 years decreases the risk of zoster, through increased access to cases of varicella

The data were also examined to investigate whether recent exposure (in the last year) to cases of VZV or children increased the risk of zoster, as has been suggested by some studies (outlined in Chapter 2).

6.1.2 Data categorisation

Contacts with cases of varicella or zoster in the last ten years were categorised simply into number of contacts, grouping categories to ensure that there were at least thirty contacts amongst controls in each category. Social exposures to children in the last ten years were categorised in stages. First, exposures were divided into three types of contact:

1. Contact with specific children living in the household

2. Contact with specific children not living in the household (e.g. grandchildren and neighbours)

3. Contact with a range of different children in groups with changing membership (e.g. at school playgrounds or parties).

Secondly, the number of social contacts in each group was calculated by multiplying average frequency of contact (per week or per month) by duration of contact (in years) for each child contacted, and summing the results. Thirdly, the total number of contacts was categorised as 'none' and into two, three or five quantiles of exposure, based on the number of exposed controls and the distribution of exposure amongst them.

Occupational exposure to children was identified from the job calendar. These occupations were divided into a further three types of contact:

4. Contact with a few specific children through childcare (e.g. childminding, full-time parenting)

5. Contact with multiple well children, (e.g. teachers)

6. Contact with multiple ill children, (e.g. doctors)

It was not possible to calculate the total number of occupational child contacts from the job calendar, and so duration of occupational exposure in the last ten years was used. This was categorised as 'none', 'up to 5 years' and 'more than 5 years'.

These data were used to create three levels of exposure for use in a hierarchical model:

- Exposure to *varicella cases* or *zoster cases*

- Exposure to *multiple children* (2, 3, 5 and 6, above)

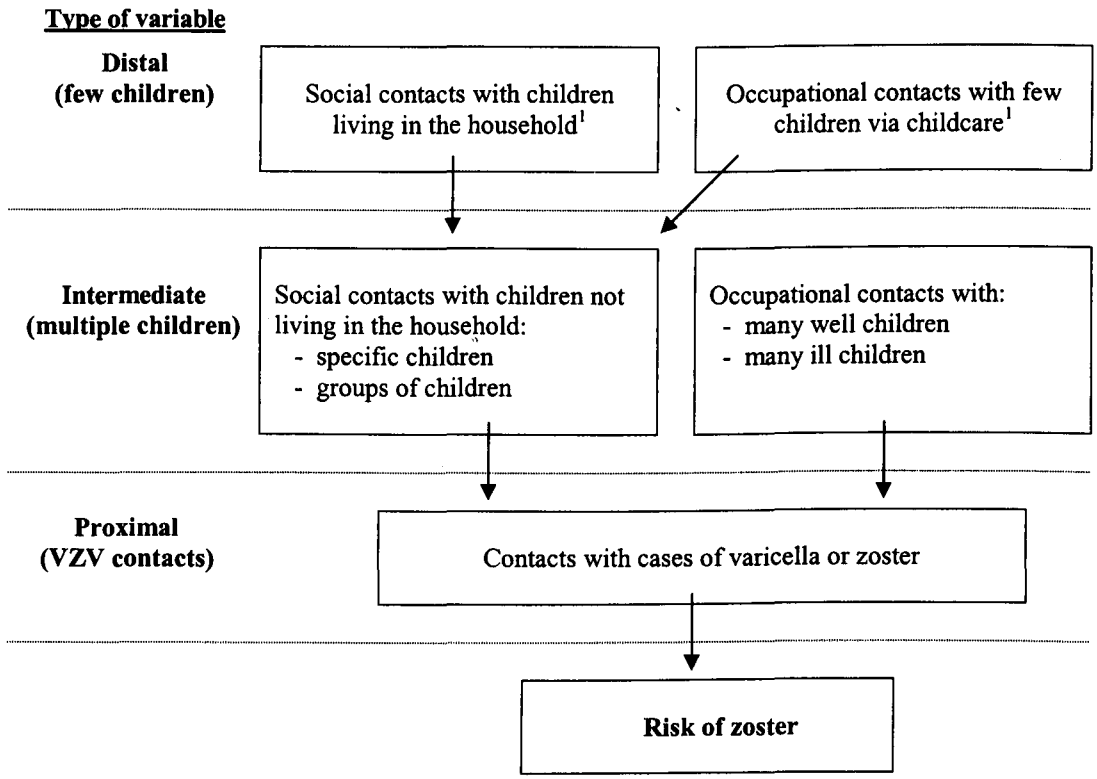
- Exposure to *a few children* (1 and 4, above)

6.1.3 Modelling strategy

A conceptual framework was used to explore the interrelationship of child and VZV (varicella or zoster) exposures in the last ten years and in the last year.[349] Variables were classified as distal, intermediate or proximal, according to their position in the proposed

chain of causation (see **Figure 6.1**, below). In this framework, contacts with cases of varicella or zoster were assumed to have a direct effect on the risk of zoster, and were categorised as proximal variables. Social or occupational contacts with *multiple* children likely to result in varicella exposures were categorised as intermediate variables. Contacts with *a few* children living in the household or via childcare work were categorised as distal variables, because some of their effect might be mediated through contacts with a wider range of children outside the household (the intermediate variables). Distal variables were added first to the multivariable model, and retained as long as they remained significantly associated with zoster ($p \leq 0{\cdot}1$). Intermediate variables were added second, to demonstrate the extent to which they explained the effect of distal variables, then proximal variables were added to determine whether they explained distal and intermediate factors. Variables excluded at the univariable or distal stages of analysis were added again at the proximal stage, to assess whether they became significantly associated with zoster in the presence of other variables. Potential confounders were added to the model as outlined in Chapter 3, and effect modification of contact variables by age was investigated.

Figure 6.1: Conceptual framework for modelling the effect of contacts with children or with cases of varicella or zoster on the risk of zoster



[1] Parents who stayed at home to look after their children full-time appear in both distal groups

6.1.4 Univariable analyses

Contact with one or more case of either varicella or zoster in the last ten years was strongly associated with protection against zoster (OR= 0.52, 95% CI = (0.37-0.72) p=0.0001). When contact with varicella cases was considered as an ordered categorical variable, there was strong evidence of a dose-response effect (**Table 6.1.1**). Contact with zoster cases also demonstrated graded protection, but this was less strongly significant.

Exposure to children in the last ten years was also associated with protection against zoster. Protection increased with longer duration of occupational exposure to multiple ill children or to a few children via childcare, and with greater numbers of social contacts with children in various settings (**Table 6.1.2**). However, there was no significant association between duration of occupational exposure to multiple well children in the last ten years and risk of zoster, even after analyses were restricted to individuals working in primary school or nursery settings (OR=0·94; 95% CI=0·47-1·87, p=0.861). Similar effects were obtained when lifetime occupational exposures in various settings were examined (data not shown).

6.1.5 Multivariable analyses

Exposures in the last ten years: Neither distal child contact variable remained significantly associated with risk of zoster after adjusting for the effects of intermediate social child contacts (**Table 6.1.3 and Figure 6.1**). Childcare and household contact variables were therefore dropped from the model. Intermediate social and occupational child contact variables remained significantly associated with protection against zoster in the intermediate model after adjusting for each other, with little change to the effect estimates (data not shown). However, the strength of associations decreased after adjusting for contact with known cases of varicella, remaining most strongly significant for contacts with children in groups (**Table 6.1.4**, column 3).

Contact with varicella cases remained strongly associated with protection against zoster after adjusting for occupational and social child contacts (**Table 6.1.4**, column 3, bottom half). In contrast, contact with zoster cases was not significantly associated with zoster in the final model. Ethnicity slightly confounded the effect of occupational exposure to ill children, and was added to all models. After adding ethnicity, other potential confounders (childhood residence in the tropics and socioeconomic variables) made minimal difference to effect estimates for the variables of interest. The effect of the contact variables did not vary with participants' age (p for interaction > 0.3 for all).

Study participants were not tested for human immunodeficiency virus (HIV) infection, and the effect of child contacts might be confounded by undiagnosed HIV infection in cases. Homosexual men in London are a group at high risk of HIV infection (increasing their chance of developing zoster), and may have relatively few child contacts.[115,284] Multivariable analyses were therefore repeated in two subgroups of individuals at low risk of HIV infection, 1) women and 2) all individuals aged more than sixty years. Statistical power was reduced, but protective trends associated with social and occupational child contacts were similar to those demonstrated in the whole dataset (**Table 6.1.5**, columns 3 and 4). The effect of imperfect specificity of the 'probable' zoster case definition was also investigated by repeating analyses in the subset of 'confirmed' cases and their matched controls. Similar protective patterns were again demonstrated (**Table 6.1.5**, column 5).

Exposures in the last year: Similar protective effects were found when the analysis was repeated for child and VZV contacts in the year before interview (data not shown). Household child contacts and childcare work in the last year were no longer significantly associated with zoster in intermediate models. Contacts with specific children living outside the household, with children in groups and with ill children were protective in intermediate models, and effects decreased after adjustment for varicella contacts in the last year. Contacts with children in groups remained most significantly associated with zoster in the final model (p=0.039); individuals with ≥313 contacts in the last year had less than two-fifths the risk of zoster compared to those with no contacts (OR=0.37; 95% CI=0.16-0.86). Adjustment in this model for the effect of past child contact exposures was limited, as some of these were correlated with recent child exposure variables and could not be added to the model. However, recent varicella contacts were weakly associated with risk of zoster in the final model after adjustment for past varicella contacts (p=0.041); compared to individuals with no known varicella contacts in the last year, those with one contact were at increased risk of zoster (OR=1.97; 95% CI=1.03-3.79), and those with two or more contacts tended towards a decreased risk of zoster (OR=0.57; 95% CI: 0.23-1.42). Similar results were obtained from analyses of social contacts with children or with cases of varicella in the month before rash onset in the case (data not shown).

The effect of social contacts in the last year with specific children living outside the household varied significantly with age (p for interaction=0.014), with protection against zoster restricted to individuals aged less than 60 years (**Table 6.1.6**). Older individuals were in contact with fewer children compared to younger individuals but saw these few children more frequently, resulting in repeated contacts with the same children. This may

have resulted in fewer opportunities to meet children with varicella. The joint importance of contacting a range of different children and the frequency of these contacts was investigated by comparing the effect of frequency of contact (mean number of contacts per child in the last year) amongst individuals contacting few (1-3) or many (4-27) specific children living outside the household (**Table 6.1.7**). Results of adjusted analyses indicated that increasing frequency of child contact was protective against zoster only amongst individuals contacting at least four different children in the last year (*p* for interaction=0.073).

6.1.6 Discussion

The findings suggest that continued exogenous exposure to varicella is protective against zoster in latently-infected adults. This is consistent with the report by Gershon *et al* that leukaemic children were significantly less likely to develop zoster if they had household exposure to varicella, and that many of the protected children developed increased VZV-specific immunity, demonstrating exogenous boosting.[108] The present analyses have also received subsequent support from a recently published study by Brisson *et al.*[350] This comprised an analysis of the 4th MSGP study, which for the first time collected data on the household composition of patients.[23] Brisson *et al* found that patients who lived with a child were at significantly lower risk of developing zoster in the year of the study (RR=0.75, 95% CI=0.63-0.89), and estimated that the duration of protection associated with this exposure lasted on average for 20 years (95% CI=7-41 years).

In this study, analyses using a hierarchical model building strategy demonstrate that living with children appears to protect against zoster largely by increasing access to a range of other children outside the household, and that the protection afforded by contacts with multiple children appears to be largely explained by contacts with varicella cases. The latter conclusion is supported by analyses showing that protection against zoster is strongest where contacts are with children in groups of changing membership in occupational or social settings (increasing the likelihood of contacting a case of varicella), and that protection depends on both the number of different children contacted and the frequency of contact with them.

Some protective effect of child contacts remained after adjustment for known varicella contacts. This may represent unrecognised or forgotten contacts with children with varicella, especially likely for social contacts with children in groups of changing membership. If this is so, the total protective effect of (known and unknown) varicella

contacts will be greater than that estimated in the final model, which represents only the effect of known varicella contacts independent of the effect of unknown contacts. Interestingly, occupational contact with multiple well children (e.g. teaching) was not protective against zoster. Possible reasons for this include:

1. *The nature of the contact:* perhaps varicella contacts are more distant in these settings compared to social settings, or are more limited in duration if children with varicella are absent from school whilst experiencing rash.

2. *Unmeasured differences in occupational contact between cases and controls:* there was some evidence that amongst the 40 individuals who had worked in primary school or nursery settings in the last ten years, cases may have had less frequent contacts with children compared with controls. Ten of the 13 cases either did not work regularly in the classroom (for example, working as a handyman), or had relatively little time with children (for example, violin teachers working one day a week or Heads of Department with limited classroom teaching), compared to 10 of 27 controls.

3. *Negative confounding:* any protective effect of occupational exposures with well children may have been masked by a strong confounder, such as recent stressful events. This is discussed further in Section 6.8, later in this Chapter.

Contact with zoster cases was not associated with protection against zoster. This is not surprising, as zoster is less infectious than varicella and most reported zoster contacts had rash on non-exposed areas of the body.

The independent effect of recent varicella exposure on the risk of zoster is less clear. Child contacts in the last year were similarly protective, but it was not possible to control for the effect of all past child contacts, so some recent protection may be due to past exposures. Interestingly, a single contact with a varicella case in the last year was weakly associated with an increased risk of zoster, although this was not seen with multiple contacts. It is likely that this is not a real effect, but was due to recall bias on the part of cases. Many individuals with zoster in the study believed that zoster resulted from contact with cases of varicella or zoster, and had spent time trying to remember any contacts that might have 'infected' them.

Ethnicity is a potential confounder of the effect of child contacts on risk of zoster, as some ethnic groups may be at lower risk of zoster and have greater contacts with children via extended families.[80] However, neither ethnicity nor country of residence in childhood accounted for the protective effect of child contacts in this study. Secondly, subgroup analyses

indicated that the protective effect of child contacts was unlikely to result from undetermined HIV infection or misdiagnosis of zoster cases.

Reverse causality is also unlikely to explain the findings. Firstly, the majority of cases were interviewed within two weeks of rash onset. Secondly, the number of child contacts was calculated using the average frequency before onset of rash, not the frequency in the last few days. For example, a case who saw her grandchild on average once a week in the last year would be assigned 52 child contacts, even if she had not seen the child since onset of rash. In contrast, recall bias (as outlined above) may have led to underestimation of the protective effects of child and varicella contacts, because cases may have been more likely to have remembered recent contacts compared with controls. Potential bias introduced by selective reporting of cases by practice or participation bias amongst controls is discussed in the next Chapter.

These analyses were presented at the Conference of the International Epidemiological Association (Oxford, September 2001), and at the 7th Meeting of the European Working Group on Varicella (Warsaw, November 2002). The findings have subsequently been published in the *Lancet*[351] (see **Appendix 8**).

6.2 ETHNICITY AND COUNTRY OF BIRTH

6.2.1 Specific hypotheses

The primary hypothesis was that ethnicity is related to risk of zoster, with individuals of Afro-Caribbean or Asian ethnicity at lower risk of zoster compared to individuals of other ethnicities. Secondary hypotheses comprised three possible explanations for any protection associated with ethnicity, namely that:

1. Individuals of Afro-Caribbean or Asian ethnicity have reduced exposure to varicella in the first ten years of life due to childhood residence in a 'late-varicella' country, and are more likely to acquire varicella in adolescence or adulthood. Therefore, they have a shorter duration of latent VZV infection compared to age-matched individuals who spent their childhood in 'early-varicella' countries. Shorter duration of latent infection may be associated with higher levels of VZV-specific immunity and thus lower risk of VZV reactivation as zoster.

2. Individuals of Afro-Caribbean or Asian ethnicity experience frequent exogenous boosting of VZV-specific immunity, due to multiple contacts with children in extended families.

3. Any protection associated with ethnicity is due to confounding by other protective factors against zoster, such as micronutrient intake.

6.2.2 Data categorisation

Ethnicity was categorised into four main groups – White, Afro-Caribbean, Asian and Other (mostly individuals from the Middle East and of mixed-race). For all individuals, residence in childhood was derived from the residence calendars for the ages 1-10 years, which are the ages at maximum risk for acquiring varicella in temperate countries. Residence was categorised first as a binary variable, in two ways:

1. 'Tropical' or 'non-tropical': a tropical residence was defined as lying at $\leq 25^{\circ}$ latitude North or South.

2. Early-varicella (EV) or late-varicella (LV) residences: LV residences included those within the Caribbean region, South India, Sri Lanka or South East Asia (see Chapter 2, Section 2.3.3.1).

Childhood residence in an LV country was hypothesised to represent reduced risk of acquiring varicella in childhood. However, some participants spent only a small proportion of their early childhood in LV countries and then returned to EV countries to attend primary school. As heaviest exposure to cases of varicella was likely to occur at primary school, the duration and timing of childhood residence in LV countries had to be taken into account. Therefore, individuals were divided into three groups:

1. Childhood residence solely in an EV or non-tropical country

2. Childhood residence in an LV or tropical country - mostly before primary school age

3. Childhood residence in an LV or tropical country - for most or all of primary schooling (with ≥ 4 years between the ages of 5-10 years)

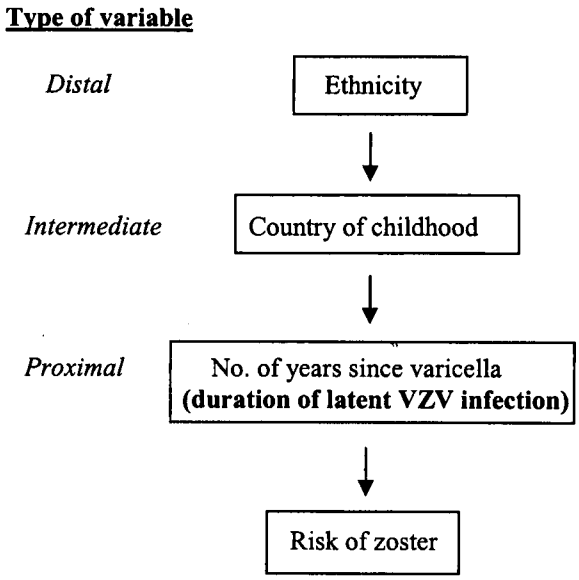
Age at varicella was categorised into four age groups that reflected differing known or hypothesised risks of zoster – 1-10 years (baseline), less than one year old (identified in previous research as a risk factor for zoster),[17,101] and 11-20 years and greater than 20 years (both hypothesised to be associated with lower risk of zoster). Duration of latent VZV

infection was derived from age at varicella and current age, and was expressed as number of years since varicella. When creating this variable, individuals who had a history of varicella at unknown age were assumed to have acquired varicella in childhood between 1-10 years of age.

6.2.3 Modelling strategy

A hierarchical approach was used to investigate the interrelationship of ethnicity, country of childhood residence and duration of latent VZV infection. This conceptual framework is outlined in **Figure 6.2**. Ethnicity was categorised as a distal variable, childhood country of residence as an intermediate variable, and duration of latent VZV infection as a proximal variable. Ethnicity was added first to the model. Country of residence in childhood was added second, to explore the extent to which it explained the effect of ethnicity. Duration of latency was added last, to estimate the extent to which this putative direct factor explained distal and intermediate factors.

Figure 6.2: : Conceptual framework for modelling the effects of ethnicity, country of childhood and age at varicella on risk of zoster



The hypothesised mediation of the effect of ethnicity through increased social child contacts and the confounding effects of other variables were examined later, in the combined model (described in Section 6.8, below).

6.2.4 Univariable analyses

Results of univariable analyses are summarised in **Table 6.2.1**. There was no overall significant association between risk of zoster and ethnicity. However, individuals of Afro-Caribbean ethnicity were at significantly lower risk of zoster compared to white participants (p=0.039).

In all, 61 individuals spent at least part of their childhood in a tropical country, and 47 individuals spent part or all of their childhood in an LV country – seven (1%) White, 30 (60%) Afro-Caribbean, six (38%) Asian and four (21%) participants of 'Other' ethnicity. Of these 47 individuals, 38 spent their entire childhood in the LV country and two spent their primary school years there. The remaining seven individuals had shorter duration of childhood residence, five leaving before attending primary school (after 1.5-3.2 years of residence), one living in India from birth until aged 6.2 years, and one living in Singapore between the ages of 4.5 and 6 years. There was no significant association between risk of zoster and duration of residence in childhood either in a LV country or in a tropical country (**Table 6.2.1**). Similar results were obtained when residence in an LV or tropical country was recoded as a binary variable, with the intermediate category grouped either with the baseline or with the third category. Effect estimates were also similar when LV countries were restricted to those with strongest evidence of late-onset varicella (the Caribbean region, South India and Sri Lanka, data not shown).

Sixty-five percent of participants remembered having varicella in the past. A history of varicella was significantly associated with protection against zoster (OR=0.68, 95% CI=0.48-0.98, p=0.037).. Age at varicella was weakly associated with risk of zoster - individuals who acquired varicella before the age of one year were at a non-significantly increased risk of zoster, and those who acquired varicella after ten years of age were at non-significantly lower risk (**Table 6.2.1**). After re-categorising individuals who had varicella at unknown age as having acquired varicella between the ages of 1-10 years, there was evidence that shorter duration of latent VZV infection (fewer years since varicella) was associated with protection against zoster. This graded protection was demonstrated more clearly after individuals with no history of varicella were excluded (**Table 6.2.1**, last column).

The associations between ethnicity, country of childhood, and age at varicella were also examined amongst controls, to see whether the hypothesised relationships between the variables existed in the population from which the cases arose. A history of varicella was

not significantly higher amongst White controls compared to Afro-Caribbean controls (68.4% vs. 57.5%, p=0.153). Similarly, there were no significant differences between White and Afro-Caribbean controls in age at varicella (p=0.751). However, 86.6% of controls (of any ethnicity) who attended primary school mostly or entirely in an EV country acquired varicella before the age of ten years, compared to 64.3% of controls who attended primary school in an LV country (p=0.02).

6.2.5 Multivariable analyses

After adding LV residence in childhood to the model, the effect of Afro-Caribbean ethnicity was slightly more protective and the effect of Asian ethnicity was reduced by about 10% (data not shown). However, childhood residence was not retained in the model, because it was not significantly associated with risk of zoster on univariable or multivariable analyses (p>0.2), was strongly correlated with ethnicity (r=0.439), and resulted in marked increase in standard errors of the effect estimates. Similar results were obtained when tropical residence in childhood was used instead of LV residence. The importance of country of childhood as a factor on the causal pathway between Afro-Caribbean ethnicity and zoster was further examined by restricting analyses to the 682 people who spent their childhood in the UK or another EV country. The effect of Afro-Caribbean ethnicity in this reduced dataset was very similar to the overall effect estimate (OR=0.40, 95%CI=0.13-1.29).

Adding the duration of latent VZV infection as a proximal variable to the model increased the risk associated with Asian ethnicity, but had little effect on other estimates (**Table 6.2.2**). Afro-Caribbean ethnicity and recent acquisition of varicella remained significantly associated with protection against zoster in the proximal model. Analyses were repeated after restricting data to cases and matched controls with a history of varicella (n=353); similar results were obtained (**Table 6.2.3**). Results were also similar when ethnicity was recoded as a binary variable (Afro-Caribbean and other, data not shown)

There was no evidence that the effect of ethnicity or time since varicella varied with age (p>0.3 for both, data not shown).

6.2.6 Discussion

In this study population, individuals of Afro-Caribbean ethnicity were at lower risk of zoster compared to individuals of White ethnicity, but Asian ethnicity was not significantly associated with risk of zoster. A higher proportion of Afro-Caribbean participants spent their

childhood in an LV country compared to Asian participants (60% vs. 38%), but childhood residence did not appear to explain the protection associated with Afro-Caribbean ethnicity. Categorisation of childhood residence as an LV location was imperfect, as data on average age at varicella is scarce for many regions, and might vary within countries due to factors such as rural or urban residence and social mixing patterns (as outlined in Chapter 2, Section 2.3.3.1) . However, the results of the proximal model suggest that the protective effect of Afro-Caribbean ethnicity was not mediated through late acquisition of varicella (see **Table 6.2.2**).

Duration of latent VZV infection (number of years since varicella) remained significantly associated with zoster in the proximal model. This supports the hypothesis that waning of VZV-specific immunity with increasing time since varicella predisposes to zoster. The effect of duration of latent VZV infection might be expected to vary with current age, with a weaker effect seen amongst younger individuals. The lack of demonstrable effect modification may have been because there were only a few younger individuals with long latency and older individuals with short latency. The suggestion of an increased risk of zoster amongst individuals who acquired varicella in the first year of life is consistent with previous reports, although numbers were very small and the result did not reach statistical significance.[17,101]

Many participants (including a number of confirmed cases) had no recall of past varicella. As more than 80% of these individuals were over 50 years old, this may simply be due to the length of time since varicella occurred, as has been demonstrated elsewhere.[352] One concern with this is that if some controls had not experienced varicella, they would not be at risk of zoster. However, a lack of varicella history was associated with an increased risk of zoster. Some cases may have unknowingly acquired varicella *in utero* or in infancy, increasing their risk of zoster.[17,101,102] Alternatively, some cases may have experienced very mild or subclinical varicella and so developed lower levels of protective immunity. The findings suggest that controls without a history of varicella probably acquired varicella in the past, and were at risk of developing zoster. Lack of varicella did not explain the effect of Afro-Caribbean ethnicity, which remained protective against zoster amongst individuals with a positive varicella history.

Some individuals could not remember the age at which they developed varicella. The assumption that these individuals probably acquired varicella between the ages of 1-10 years is reasonable, but may have led to some misclassification of age at varicella. If this occurred, the magnitude of protection associated with delayed varicella or the increased risk associated with very early varicella may have been underestimated.

The number of non-White participants was relatively small, and the estimates of the effect of ethnicity had wide 95% confidence intervals. Low numbers probably resulted partly from exclusion of individuals from sub-Saharan Africa, and from the fact that many individuals from ethnic minorities in South London are relatively young. This is unlikely to have introduced bias, as controls were matched to cases by age, but there were relatively few non-White individuals in the predominantly older cases and controls. Bias could have been introduced if general practitioners were less likely to report individuals of Afro-Caribbean ethnicity under the mistaken belief that they were African. However, general practitioners were not required to apply ethnic exclusion criteria, and were requested to report all cases. More African cases were reported than Afro-Caribbean cases (18 versus 10 adult cases).

The analysis demonstrates that duration of latent VZV infection is a determinant of zoster, but it does not explain the protection associated with Afro-Caribbean ethnicity in this population. Other proximal determinants of the effect of Afro-Caribbean ethnicity were examined in the combined model. As discussed later in this Chapter, much of the protective effect was explained by increased social and occupational child contacts, and the remaining effect disappeared after controlling for other factors.

6.3 FOOD INTAKE, MICRONUTRIENT INTAKE AND ANTHROPOMETRY

6.3.1 Specific hypotheses

The primary hypotheses were that:

1. Low daily intakes of specific micronutrients in the year before interview or in the two months before rash onset increase the risk of zoster. These micronutrients are vitamin A (as retinol equivalents), vitamins C, E and B_6, folic acid, iron and zinc.
2. Low intakes of foods rich in the micronutrients of interest increase the risk of zoster

The secondary hypotheses were that:

1. Individuals with illnesses associated with deficiency of any of the micronutrients of interest are at increased risk of zoster
2. Low body mass index (or mindex or demiquet as alternative measures of body mass index) increases the risk of zoster

6.3.2 Data conversion and categorisation

Individual micronutrient intake: food frequency data were first converted into energy-adjusted daily intakes of each of the micronutrients of interest, as outlined in Chapter 4 (Section 4.2). Daily intakes were calculated for two time periods:

1. Daily intake in the last year
2. Daily intake in the two months before rash onset in the case.

In this way, the effects of longer-term and recent dietary intake were assessed. Micronutrient intake derived from a) foods and b) from foods and dietary supplements combined were considered separately, to examine both the effects of usual diet and of total micronutrient intake. Energy-adjusted intakes were categorised into quintiles of intake, based on the distribution amongst controls.

Combined micronutrient intake: two new micronutrient variables were created to investigate the effects of combined micronutrient intake on the risk of zoster:

1. *Total micronutrient score:* for each of the seven micronutrients, quintiles of intake were given a score from 1 (lowest level) to 5 (highest level). Individuals' total micronutrient scores (with values lying between 7 and 35) were obtained by summing their scores for each micronutrient. The total scores were then re-categorised into quintiles of scores, with similar numbers of controls in each group. Individuals with high or low scores mostly had high or low intakes of all seven micronutrients of interest. However, relatively high scores were also found amongst individuals who had very high levels of one or two micronutrients but lower levels of others. Therefore, a second variable was created that reflected high micronutrient intakes over the range of micronutrients of interest, and not an unbalanced intake.

2. *Number of micronutrients at the highest intake level:* for each micronutrient, intake was scored as '1' for the highest quintile of intake and '0' for lower intakes. An individual's score was the number of micronutrients at the highest quintile of intake (with values of 0-7). For this variable, all individuals with high scores had relatively high intakes of most of the micronutrients of interest.

Intake of micronutrient-rich foods: fruit and vegetables were chosen to represent foods rich in the micronutrients of interest, as many of these contain relatively high quantities of vitamin

C, vitamin A (as carotenoids), folic acid and (for some vegetables) iron.[336,338] Overall frequency of consumption of fruit or vegetables was calculated by summing frequencies of individual fruit and vegetable items reported in the food frequency questionnaire. The types of fruit or vegetables considered in these analyses were progressively restricted to those fruits or vegetables with higher levels of the micronutrients of interest; as follows:

1. Fresh, frozen or tinned fruit or vegetables (of all colours)
2. Fresh or frozen fruit or vegetables (of all colours)
3. Green, yellow or orange/red vegetables - these often contain particularly high levels of vitamin C, folic acid and carotenoids.[338]

For each fruit or vegetable variable, intake was categorised into five groups. The lowest intake was pre-defined as less than one portion a week for fruit intake, and less than one portion a day for vegetable intake or fruit and vegetable intake combined. Cut-off points for higher intakes were based where possible on approximately equal numbers of controls in each group.

Underlying illnesses: a new variable was created, comprising individuals with illnesses or conditions resulting in decreased micronutrient availability and/or increased requirement, as these individuals might have a deficiency of one or more of the micronutrients of interest. In this population, such conditions included eating disorders, dysphagia with food regurgitation, ulcerative colitis, pregnancy, and epilepsy treated with phenytoin (which can result in folate deficiency).[353] Individuals who had been diagnosed as having iron- or folate-deficiency anaemia were also included in this category. A second variable was generated, comprising individuals who had been prescribed one or more of the seven micronutrients.

Anthropometric indices: body mass index was categorised into the same four groups that were used in the second National Diet and Nutritional Survey:[342] 1) 'Underweight' (20 or less), 2) 'Average' (over 20 to 25), 3) 'Overweight' (over 25 to 30), and 4) 'Obese' (over 30). Other anthropometric measures were divided into quintiles of values, based on the distribution amongst controls, with the second lowest quintile taken as the baseline.

6.3.3 Analytical and modelling strategy

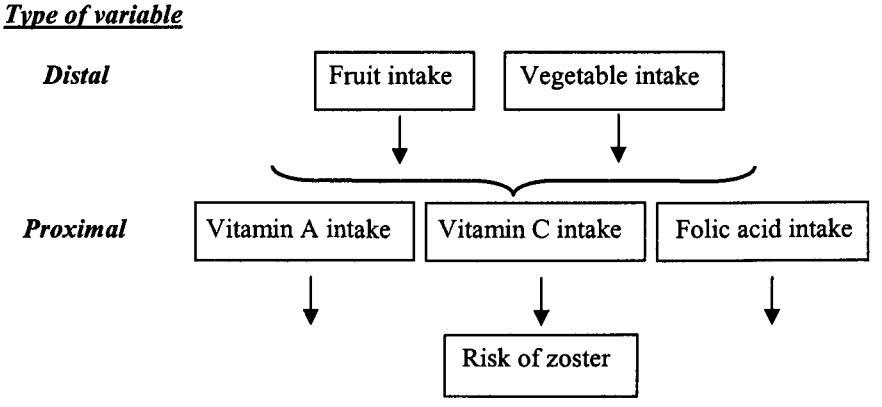
The effect of micronutrient intake from **food** was examined firstly in all individuals, and then restricted to individuals who were not taking micronutrient supplements. This was done to

ensure that any effect of food intake was not being overwhelmed by supplement intake. The effect of **total** micronutrient intake (from foods and supplements combined) was then examined. This was potentially confounded by illnesses or conditions associated with micronutrient deficiency, because 1) individuals with these conditions were potentially at higher risk of zoster and 2) some of these individuals had high micronutrient intakes from prescribed vitamins or minerals. Therefore, analyses of total micronutrient intake were carried out first on all individuals, and then repeated after excluding individuals who had underlying illnesses that could modify micronutrient availability or who had been prescribed one or more of the seven micronutrients under investigation.

A hierarchical approach was used to explore the interrelationship of the effects of fruit or vegetable intake and food intake of the commonest micronutrients found in fruit and/or vegetables - vitamin C, vitamin A or folic acid (**Figure 6.3**, below). Fruit and vegetable intake were categorised as distal variables, and vitamin C, vitamin A and folic acid intake were categorised as proximal variables. Fruit or vegetable intake was added first to the model. Micronutrient intakes were then added to explore the extent to which the effect of fruit or vegetable intake was mediated via micronutrient intake. The effect of fruit and vegetable intake was also adjusted at this stage for potential confounders including ethnicity, smoking, alcohol and energy intake, house tenure, car ownership, illnesses associated with micronutrient deficiency and intake of non-prescribed supplements containing the micronutrients of interest.

Combined micronutrient variables (described above) were used in preference to exploring multiple interactions between pairs of micronutrients. Effect modification by age was considered for each micronutrient, for combined micronutrient scores and for fruit and vegetable intake.

***Figure 6.3**: Conceptual framework for modelling the effects of fruit and vegetable intake and intake of vitamin C, vitamin A and folic acid on risk of zoster*



6.3.4 Univariable analyses

Intakes in the last year: the effects of energy-adjusted daily intake from foods of each micronutrient on the risk of zoster are summarised in **Table 6.3.1**. No significant associations were found, except for a significant trend of increasing risk associated with decreasing vitamin C intake.

A total of 283 individuals were taking non-prescribed supplements containing one or more of the seven micronutrients of interest. These individuals were at similar risk of zoster compared to non-supplement takers (OR=0.93, 95% CI=0.67-1.30, p=0.671). Analyses of intake from foods were repeated in the smaller dataset (n=306) of matched sets of individuals who had not taken micronutrient supplements. The significant trend associated with vitamin C intake was not evident in this smaller group (p for trend=0.909), and no other significant associations were found (data not shown).

When total intake from both foods and dietary supplements was examined, the effect of total vitamin C was weaker than that of intake from diet alone, with poor evidence of a dose response effect. (p for trend=0.160, data not shown). None of the other total micronutrient intakes was significantly associated with risk of zoster. This analysis included data from 24 individuals (10 cases and 14 controls) with illnesses or conditions associated with micronutrient deficiency. These individuals were at approximately three times the risk of zoster compared to the rest of the study population (OR=3.18, 95% CI=1.32-7.65, p=0.008), and the majority (16/24) had been prescribed one or more of the micronutrients of interest. One other individual who did not report a history of iron-deficiency anaemia had also been prescribed maintenance doses of iron sulphate (200mg/day). The 17 individuals who had been prescribed micronutrients were at about four times the risk of zoster compared to individuals who were not taking prescribed supplements (OR=4.13, 95%CI=1.43-11.98, p=0.006). The effect of intake from diet and supplements combined was reconsidered after excluding the 25 individuals with these illnesses and/or prescribed micronutrients. Amongst the remaining 227 matched sets with at least one case and one control (n=671), no significant associations were found (**Table 6.3.2**). Low vitamin E intake was weakly associated with risk of zoster (p=0.055), although there was no clear pattern of risk.

Neither total micronutrient score nor the number of micronutrients at the highest intake level were associated with risk of zoster when scores were derived from a) foods or b) foods and supplements combined (**Table 6.3.3**). This lack of association persisted after excluding from analyses of food intakes those individuals taking supplements, and after excluding

from analyses of food and supplement intakes those individuals with illnesses associated with micronutrient deficiency (data not shown).

The effects of intakes of fresh fruit and fresh or frozen vegetables on risk of zoster are summarised in **Table 6.3.4**. Low combined fruit and vegetable intake was associated with a strong graded increased risk of zoster; individuals taking one or less portion a day of fruit or vegetables were at nearly three times the risk of zoster compared to individuals taking at least eight portions a day. This increased risk remained when fruit and vegetable intake were considered separately. The effect estimates were higher when intake of green, red or yellow vegetables (vegetables rich in the micronutrients of interest) was analysed, although the 95% confidence intervals were wide. Effects were similar when tinned fruit and vegetables were included in the analyses (data not shown).

In order to clarify whether the lower frequency of fruit and vegetable intake amongst cases was specific for these foods or whether cases generally reported lower frequency of food consumption, analyses were repeated for other food groups. For example, there was no association between frequency of consumption of potatoes (which were not classified as vegetables in the FFQ) and risk of zoster (p=0.705, data not shown).

Intake in the two months before rash onset: the effects on the risk of zoster of 1) daily energy-adjusted intake of each micronutrient from foods 2) intake from foods and supplements combined and 3) combined micronutrient intake were all similar to the effects of intakes in the last year (**Table 6.3.5-6.3.7**). The effects of fruit and vegetable intakes in the two months before rash onset were mostly slightly weaker compared to the effects of intakes in the last year (**Table 6.3.8**).

Anthropometric indices: 17 individuals were not included in analyses because they could not stand safely on the weighing scales (12), refused to be weighed (2) or (for one case and two controls) because they were pregnant. Among the 240 matched sets with data for at least one case and one control (n=677 individuals), there was no evidence that being underweight (BMI≤20) was associated with an increased risk of zoster (**Table 6.3.9**). Similarly, there was no increased risk of zoster amongst individuals with the lowest quintiles of mindex or demiquet measurements.

6.3.5 Multivariable analyses

Table 6.3.10 summarises the results of the hierarchical models used to explore whether the effect of fruit and vegetable intake in the last year was explained by vitamin C intake in foods. Effect estimates for combined fruit and vegetable intake and for fruit intake alone were little altered after adjusting for vitamin C intake (**Table 6.3.10**, column 3). The effect estimates for vegetable intake were slightly diminished, but a significant dose-response effect remained. The effect estimates for the fruit and vegetable variables were also unaffected after adjusting for vitamin A intake or for folate intake (data not shown). In contrast, the increasing risk on univariable analysis associated with lower vitamin C intakes from food disappeared after controlling for fruit and/or vegetable intake, particularly after controlling for fruit intake (**Table 6.3.11**).

Smoking negatively confounded the effect of fresh fruit intake but not the other variables (**Table 6.3.10**, final column). The effect of fruit and/or vegetable intake was not confounded by ethnicity, house tenure, car ownership, alcohol or total energy intake, body mass index, illnesses associated with micronutrient deficiency or intake of non-prescribed supplements containing the micronutrients of interest. Analysis of fruit, vegetable and vitamin C intake in the two months before rash showed similar results (data not shown).

Effect modification by age: there was little evidence that age modified the effect of fruit or vegetable intake in the last year, or fruit intake in the two months before rash (p for interaction all >0.2, data not shown). However, there was some evidence that the protective effect of vegetable intake in the two months before rash was restricted to individuals aged 60 years or older (**Table 6.3.12**). There was also evidence amongst older individuals of an effect associated with combined micronutrient intake in the last year - there was a significant trend towards increasing zoster risk with decreasing total micronutrient scores derived from food intakes (p for interaction=0.004), and a similar trend with decreasing number of micronutrients at the highest level of intake (p for interaction=0.0007, **Table 6.3.13**). These effects in older individuals were not confounded by ethnicity, smoking, house tenure, car ownership, alcohol intake, micronutrient supplement intake or illnesses associated with micronutrient deficiency. Results were similar when analyses were restricted to individuals who were not taking micronutrient supplements, but were less strong when combined micronutrient variables were derived from both food and supplement intake (data not shown). Similar effect modification was seen for combined micronutrient intake in the two months before rash onset (data not shown).

6.3.6 Discussion

In this population, low intakes of fruit and vegetables were associated with increased risk of zoster, with a strong dose response effect. Effect estimates were higher for intake of green, red or yellow vegetables than for intake of all vegetables, consistent with the hypothesis that high intakes of vitamin C, carotenoids and folic acid may protect against zoster by maintaining functional cell-mediated immunity. As discussed in Chapter 2, a number of studies have shown that supplementation with these nutrients can have a beneficial effect on cell mediated immunity in the elderly. However, none of the individual micronutrient intakes in this study was associated with risk of zoster except dietary vitamin C. This micronutrient is mostly obtained from fruit and vegetables, and plasma vitamin C levels have been shown to be more strongly related to fruit and vegetable consumption compared to other antioxidants.[354] Results of the hierarchical analysis suggest that the effect of vitamin C intake was simply a marker for fruit and vegetable intake, and not a step on the causal pathway. Nevertheless, low combined micronutrient intake was associated with increased risk of zoster in older individuals, and people with illnesses associated with micronutrient deficiency and/or taking prescribed micronutrients were at significantly higher risk of zoster. How can these findings be reconciled?

Firstly, both frequency of fruit/vegetable intake and intake of individual micronutrients may have been estimated inaccurately, but to different degrees. Possible sources of inaccuracy include the following:

Frequency of food consumption: non-differential misclassification of frequency of consumption could have led to underestimates of both fruit/vegetable consumption and micronutrient intake. Recall bias amongst cases in underreporting fruit/vegetable consumption but not other food items could have resulted in a stronger effect of fruit/vegetable consumption compared to overall micronutrient intake. However, there was no indication that cases believed that eating fruit and vegetables protected against zoster.

Portion size: in the analysis it was assumed that individuals of the same age and sex ate similar portion sizes of each food. This was likely for most fruits and for a few other food items, where portions were easily defined as 'pieces'. However, 'medium portions' of vegetables and most other foods may have varied between individuals. This may have affected estimates in various ways. For example, if cases ate smaller portions of a range of foods compared to controls, this could have overestimated their dietary micronutrient intake (calculated using

falsely high portion sizes), leading to an underestimate of the effect of micronutrient intake on risk of zoster.

Food item chosen for each FFQ item: a single food item was chosen to represent each broadly characterised FFQ item in each age/sex group, as described in Chapter 4 (Section 4.2.3). The choice for fruit and most vegetables was narrow, as fruit and vegetable FFQ items mostly comprised a small number of specific foods. However, other FFQ items often contained a wide range of foods. For example, McCance and Widdowson lists only one food for the FFQ item 'Fresh Bananas', but 91 food items that could be included under 'Beef'.[334,336] For FFQ items comprising a range of foods, the single food chosen to represent the item may have had a different micronutrient content to the foods actually consumed by some participants, resulting in inaccuracies in their estimated micronutrient intake.

Nutrient database information: the nutrient content of specific foods is not constant, but may vary according to breed or type, farming practices, changes made by manufacturers, storage, duration of cooking, etc. Nutrient databases provide average nutrient content of foods, which may differ from actual content in some cases.

The FFQ was not used to obtain absolute values of micronutrients in the diet, but to rank individuals' intake. Nevertheless, the sources of variation described above may have led to some misclassification of ranking of micronutrient intake, and this may have been greater than misclassification of frequency of fruit and vegetable consumption.

Secondly, fruit and vegetables contain a complex mixture of nutrients, which may act together to maintain immune health. In this study, many of the cases with low fruit and/or vegetable intake ate relatively large amounts of foods such as meats and potatoes, increasing their intake of single micronutrients from other food sources. It may be that these micronutrients acting alone have relatively weak effects on the risk of zoster. High combined intake of micronutrients was associated with lower risk of zoster, although this was only demonstrated amongst older individuals. It is plausible that combined micronutrients protect against the gradual decline in cell-mediated immunity that occurs with ageing, but have less impact amongst younger individuals with generally robust immune systems. However, no effect modification of fruit or vegetable intake by age was demonstrated, except for the effect of vegetable intake in the two months before rash onset. Perhaps the mixture of nutrients in fruit and vegetables is not fully represented by the combined micronutrient variables used in the analyses, and includes substances whose effects on the immune system (of both younger and older individuals) have not yet been appreciated.

Thirdly, the apparent protective effect of fruit and vegetable intake may be due to residual confounding by other variables that affect cell-mediated immunity. A number of confounders were taken into account in the analysis, including ethnicity, smoking, alcohol intake and underlying illness. The effects of fruit and vegetable intake also remained after adjustments for other factors in the final model, described later in this Chapter. However, misclassification of some of these confounders or the presence of other unidentified lifestyle or other risk factors for poor immune health may have contributed to some of the apparent effect of fruit and vegetables. In the UK Government's Health Survey for England (HSE) 2001, low fruit and vegetable consumption was strongly associated with low household income, but it is unclear what proximal determinants of zoster this could represent.[355] Other determinants of low fruit and vegetable consumption in the HSE survey (cigarette smoking, age, sex, alcohol consumption, children living in the household, and body mass index) were either adjusted for in the present analysis or did not confound fruit consumption. One variable that might provide an alternative explanation for the effect of fruit intake is physical activity, as moderate exercise can be associated with both heightened immune functioning and with fruit intake.[356]

It is interesting that total micronutrient intake (including supplements) had less effect on risk of zoster than intake from diet alone, even after controlling for the effect of illnesses associated with micronutrient deficiencies. Perhaps some of the individuals taking micronutrients did so to compensate for poor diet or for feeling unwell, and had undiagnosed micronutrient deficiencies associated with poor immune function. The effect of fruit and vegetable intake in the two months before rash onset in the case was also less strong than intake in the year before interview. This suggests that longer-term dietary habits have a stronger effect on cell-mediated immunity compared to recent diet. Alternatively, the weaker effect may have been due to the delay between interviewing cases and controls in some matched sets. Individuals were asked about usual and seasonal diet without asking them about a specific time period, and intake in the two months before rash onset was calculated subsequently. Nevertheless, the period of interest was immediately before interview for cases, but was sometimes much earlier for controls. This could have led to differential misclassification of fruit and vegetable intake.

6.4 EXPOSURE TO ULTRAVIOLET RADIATION

6.4.1 Specific hypotheses

The analyses in this thesis focus on the effects of recent ultraviolet radiation (UVR) exposures, and UVR exposures in childhood (which might 'programme' the developing immune system). The primary hypotheses were that:

1. Individuals with high *cumulative* UVR exposure from sunlight in childhood or in the last year are at increased risk of zoster.
2. Individuals with high *intensity* of UVR exposure from sunlight in childhood or in the last year are at increased risk of zoster
3. Individuals with high *intermittency* of UVR exposure from sunlight in childhood or in the last year are at increased risk of zoster.

The three different patterns of UVR exposure (cumulative exposure, intensity of exposure and intermittency of exposure) are discussed in Section 6.4.2, below.

The secondary hypotheses were that:

1. The effects of UVR exposures on risk of zoster may be greater in individuals with a propensity to burn and/or an inability to tan compared to other individuals.
2. Hats and protective clothing protect individuals from the effects of UVR exposures.
3. Use of sunbeds and/or medical UVR exposures in childhood or in the last year increase the risk of zoster

6.4.2 Patterns of UVR exposure, data conversion and categorisation

As outlined in Chapter 2, studies of the effects of UVR exposure on the human immune system have mostly focused on the effects of short-term UVR exposure of varying dosage. Daily doses in these studies ranged from less than 1 MED to 4 MED, often administered as a short, intense exposure (see **Table 2.2**). Studies have established *cumulative* (total) UVR exposure as a risk factor for skin cancer, and this was therefore considered in this study as a potential risk factor for zoster. However, cumulative UVR dosage does not necessarily reflect *intensity* of UVR exposure, because individuals vary in the time they spend outdoors, both at their place of residence and on holiday. For example, a cumulative holiday UVR exposure of 14 MED

might include individuals with sub-erythemal doses of 0.5 MED per day during 28-day holidays, and individuals with exposures of 2 MED per day on 7-day holidays. As the risk of immune suppression (and therefore risk of zoster) may increase with intensity of exposure, UVR intensity was also examined as a risk factor for zoster. Finally, it is unclear whether the immune system adapts to continuous high doses of UVR. If so, *intermittency* of UVR exposure may be a risk factor for zoster – acute exposure to high UVR dosage in individuals with habitually low UVR exposure may increase the risk of immunosuppression. Therefore, intermittency of exposure was also investigated.

The three patterns of UVR exposure from sunlight were generated as described below, and as summarised in **Figure 6.4** (overleaf):

Cumulative UVR exposure: for non-holiday periods, total weekly UVR exposure was estimated as outlined in Chapter 4 (Section 4.1), using data on ambient UVR levels and cloud cover for each residence, the time spent outdoors between 9am and 5pm on work (or school) days and non-workdays, and the number of work (or school) days per week. For holidays, UVR exposure was derived from the ambient UVR levels and cloud cover for each holiday destination, time spent outdoors and duration of the holiday.

In childhood, UVR exposure mostly resulted from time spent outdoors in the warmer months (April-September). Most of this exposure occurred on school-days and non-school days at home, but some individuals also had briefer periods of (often intense) UVR exposure on summer holidays. However, individuals typically had very little UVR exposure during the cooler months (October to March) of childhood, either at home or on holiday. Therefore, cumulative UVR exposure in childhood was estimated only for the warmer months, as follows:

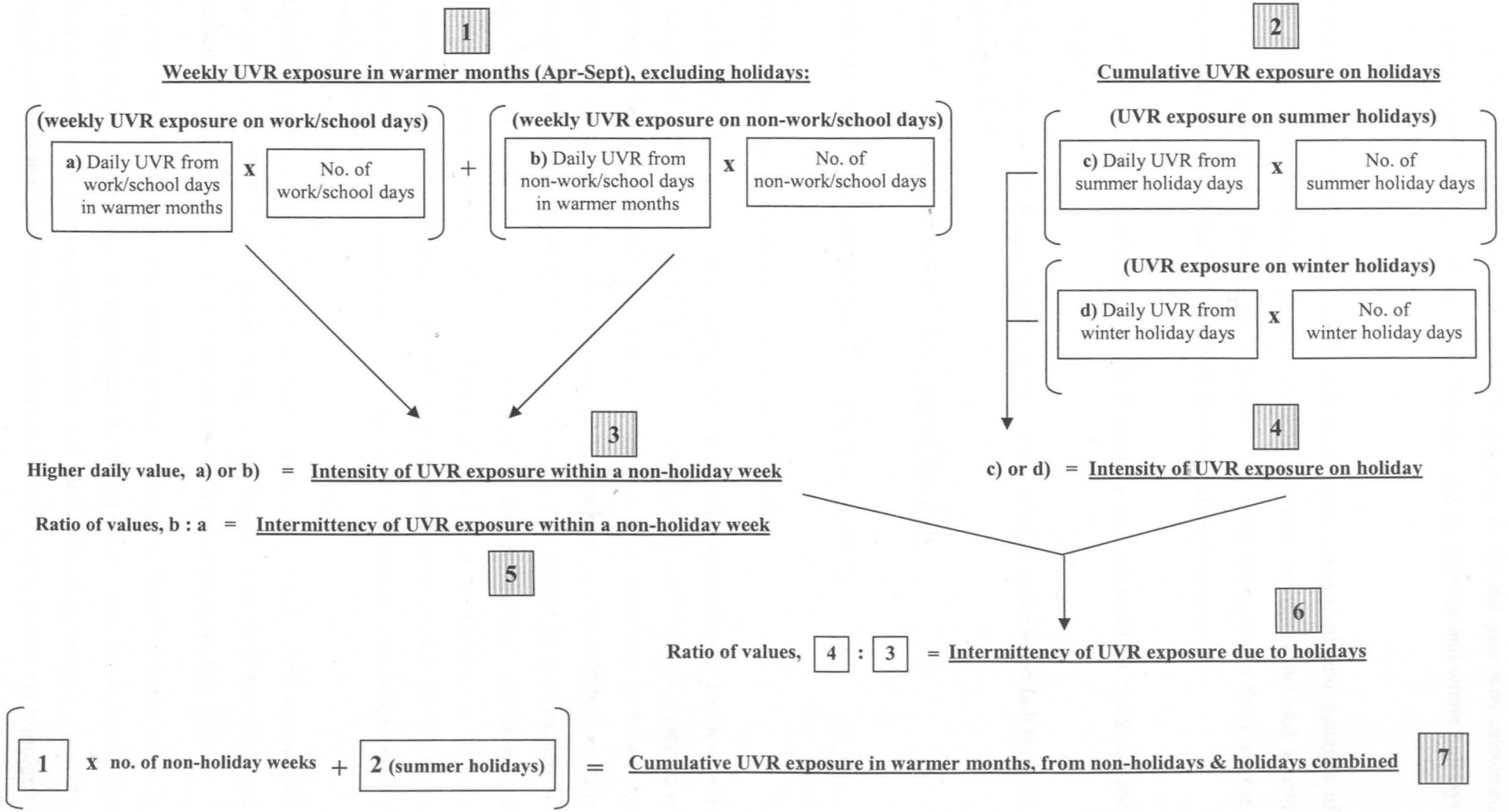
a) Exposure for the entire period of April-September, combining total non-holiday exposure (weekly non-holiday exposure x number of weeks not on holiday) with holiday exposure (**Figure 6.4,** 7)

b) Weekly non-holiday exposure in the warmer months – exposure on schooldays and non-schooldays combined (**Figure 6.4,** 1)

c) Exposure during summer holidays away from home (**Figure 6.4,** 2)

Adult UVR exposure in the last year was estimated as above, for a) the entire warmer months (non-holiday and holiday exposure combined), and for b) weekly non-holiday exposure in the warmer months (exposure on workdays and non-workdays). Although most adults had very little UVR exposure during winter months at home, some experienced sizeable UVR exposure

Figure 6.4: Generation of seven UVR exposure variables - cumulative UVR dose (1, 2, 7), UVR intensity (3, 4) and UVR intermittency (5, 6)



during winter holidays. Therefore, holiday UVR exposure in the last year was calculated separately for summer holidays and for winter holidays, and for summer and winter holidays combined.

After UVR exposures had been estimated, individuals were categorised into quintiles of exposure, based on the distribution amongst controls. Holiday exposures included a sixth category of individuals who did not go on holiday. For UVR exposures in the last year, 'holidays' included work trips that included periods of recreational sun exposure.

Intensity of UVR exposure: maximum daily UVR intensity was examined for childhood and for the last year, as:

a) The highest daily UVR dosage received in the warmer months within a non-holiday week (**Figure 6.4,** 3)

b) The daily UVR dosage received on holiday (**Figure 6.4,** 4)

Individuals were then categorised into quintiles of exposure, as above.

Intermittency of UVR exposure: this was examined as follows:

a) Intermittency within a non-holiday week: individuals who worked mostly had few non-workdays and experienced higher levels of UVR exposure on non-workdays compared to workdays. Therefore, intermittency of exposure within a non-holiday week in the warmer months was estimated as the ratio of daily UVR exposure on non-workdays to daily exposure on workdays (**Figure 6.4,** 5). For individuals who worked for less than half the week, UVR exposure on workdays was taken as the 'intermittent' exposure, and intermittency was estimated as the ratio of daily UVR exposure on workdays to exposure on non-workdays. For individuals who did not work, intermittency within the week was categorised as '0'. For childhood UVR exposures, schooldays were substituted for workdays.

b) Intermittency due to holidays: this was estimated as the ratio of daily UVR exposure on holidays to the highest daily exposure during a non-holiday week, and was calculated separately for holidays taken in the warmer and the cooler periods (**Figure 6.4,** 6).

c) Sunburns: the effect of sunburns causing severe erythema or blistering in the last year was investigated as a marker of intermittent UVR exposure. The interview did not include a question on sunburns in childhood, and most individuals did not report the timing of sunburns in earlier life, so the effect of childhood sunburns was not examined.

In addition to these three patterns of UVR exposure in childhood and in the last year, two other aspects of exposure were considered:

1. *Sunbathing:* individuals were asked at interview whether they sunbathed during non-holiday periods. This provided a marker of regular sun-seeking behaviour in adulthood, and represented acute, usually intense UVR exposure. Therefore, the effect of UVR exposure from sunbathing in the last year was examined as a single variable, irrespective of other UVR exposures.
2. *Exposure in the month before rash onset:* the possibility that high levels of UVR exposure might lead to immediate VZV reactivation were examined by analysing the effect of UVR exposure in the month before rash onset in the case, a) from non-holiday and holiday exposure combined, b) from holiday exposure alone and c) from sunbathing.

6.4.3 Analytical strategy

UVR dose-response: Kricker *et al* demonstrated that for skin cancer, the relationship between cumulative UVR exposure and risk of disease is not linear - the risk increases initially with increasing exposure, but decreases at highest exposure levels.[287] In order to explore whether this departure from linearity was also applicable to zoster, models with a quadratic term for categorical variables were compared to models with simple linear terms, using likelihood ratio tests (LRT).

Independent effects of UVR exposure variables: The independent effects of 'usual' (non-holiday) and holiday UVR exposure were investigated by adding both variables to a multivariable model. Possible negative confounders of these effects included:

a) Birth in a late varicella (LV) country: this might be associated with lower risk of zoster and high UVR exposure in childhood;

b) Current illness: this might be associated with increased risk of zoster and lower recent UVR exposure;

c) Susceptibility to burn/ability to tan - individuals with poor skin tolerance to UVR exposure often have low UVR exposure because they avoid the sun. However, they may be at higher risk of skin erythema (have a lower personal MED) at a given level of UVR exposure compared to individuals with good skin tolerance to UVR. Therefore, at any 'standard MED' dosage they may be at increased risk of immune suppression.

The potential negative confounding effects of these variables were checked in all appropriate models. The confounding effect of ethnicity was also considered, as a composite of birth in a LV country and non-white skin colour.

Effect of baseline UVR dosage on intermittency: the univariable effect of intermittency of UVR exposure takes no account of individuals' usual (baseline) level of exposure, which is used as the denominator in estimates of intermittency. Baseline UVR dosage could confound the effect of intermittency because individuals with a very low baseline levels and modest 'intermittent' levels may have low **total** levels of UVR exposure but high **intermittency** of exposure. Therefore, the effect of intermittency independent of the baseline UVR dosage was examined by adding the baseline dosage to multivariable models. In addition, individuals with low UVR baseline dosage (who are least likely to have adapted to the immunosuppressive effect of UVR) might be more vulnerable to the effects of intermittency of UVR. This was investigated by subdividing individuals into two levels of baseline exposure, and examining the effect of intermittency separately in each group. The cut-offs for categories of baseline exposure and for intermittency variables were chosen to allow sufficient numbers for at least three intermittency categories in each of the two baseline groups.

Effect of age: the effects of UVR exposures might be different in older individuals with declining cell-mediated immunity compared to younger individuals with more robust immune function. The effects of the UVR exposures outlined above were therefore investigated separately in individuals aged less than 60 years and those aged 60 years and older.

Protective effects of hats/clothing: hats and clothing may protect against the effect of UVR exposure on zoster by attenuating UVR dosage. The confounding effects of wearing hats and protective clothes were first examined in multivariable models of UVR exposure. However, some individuals living or holidaying in cooler climates wore hats and clothes to shield themselves against the cold and rain, and not to protect against the sun. Therefore, the independent effects of hats and clothes were considered separately for low and high levels of UVR exposure.

Effect modification by skin type: as discussed above, individuals' skin type may reflect their personal susceptibility to UVR-mediated immune suppression. The effects of UVR exposures were examined separately in individuals according to:

a) *Their skin response to initial UVR exposure:* individuals were divided into two groups - those who tanned with/without mild burning, and those who developed a painful sunburn with peeling or blistering;

b) *Their skin response to repeated UVR exposure:* individuals were also divided into two groups - those who obtained a moderate or deep tan, and those who obtained a slight or no tan (with or without freckling).

6.4.4 Univariable analyses (Tables 6.4.1-6.4.6)

Exposures in childhood: increasing total *cumulative* UVR exposure in the warmer months (holiday and non-holiday UVR exposures combined) was associated with a non-linear increasing risk of zoster, but this association was not significant (p=0.401, **Table 6.4.1**). However, both non-holiday UVR exposure in the warmer months and total summer holiday exposure were significantly associated with increased risk of zoster. The effect of non-holiday UVR dosage appeared to have a quadratic pattern of risk (p=0.002, **Table 6.4.1**), with strong evidence of a departure from linearity (p=0.0006 for LRT of quadratic vs. linear model). The effect of childhood holiday exposure showed a weaker quadratic relationship, with less evidence of departure from linearity in the smaller dataset of holidaymakers (p=0.148 for LRT of quadratic vs. linear model). Individuals who did not go away on holiday in childhood were at more than twice the risk of zoster compared to individuals with the lowest holiday UVR exposures, but were not at increased risk of zoster compared to all holidaymakers (OR=0.94, 95%CI=0.66-1.32, p=0.703).

The effect of *intensity* (maximum daily dose) of UVR exposure in childhood within a non-holiday week and on summer holidays showed a similar pattern to those for cumulative UVR exposures, but were less strongly statistically significant (**Table 6.4.1**). *Intermittency* of UVR exposure in the warmer months was not associated with increased risk of zoster on univariable analysis (**Table 6.4.2**) - neither high non-holiday intermittency (non-schoolday compared to schoolday exposure) nor holiday intermittency (holiday compared to non-holiday exposure) was significantly associated with zoster risk.

Few individuals (9% of cases and 11% of controls, n=603) wore hats at least half the time in childhood to protect against the sun when not on holiday, and even fewer wore protective clothes at least half the time (3.7% of cases and 3.7% of controls, n=626). Neither variable was significantly associated with risk of zoster (hats: OR=0.83, 95%CI=0.48-1.45, p=0.517; clothes: OR=1.00, 95%CI=0.41-2.41, p=1.0). Amongst holidaymakers with available

information, 22 (15.6%) cases and 53 (19.7%) controls wore hats at least half the time (OR=0.67, 95%CI=0.34-1.32, p=0.241), and two (1.4%) cases and seven (2.6%) controls wore protective clothing at least half the time (OR=0.78, 95%CI=0.14-4.36, p=0.775)

Twenty-two individuals (eight cases and 14 controls) reported medical UVR exposures in childhood, mostly in solaria set up for 'peaky' children in the 1930s. There was no association between these childhood UVR exposures and zoster risk (OR=1.16, 95% CI:0.46-2.89, p=0.753).

Exposures in the last year: high cumulative total (holiday + non-holiday) UVR exposure in the warmer months was associated with a weakly significant, near-linear increasing risk of zoster (**Table 6.4.3**). However, there was no significant association between risk of zoster and other cumulative UVR exposures in the last year, including non-holiday UVR exposure in the warmer months, total UVR exposure during summer holidays (other than a weak quadratic association), winter holidays, or summer and winter holidays combined. Individuals who did not go on summer holidays were at a non-significantly increased risk of zoster compared to individuals with the lowest quintile of UVR holiday exposure, but were not at increased risk of zoster compared to all summer holiday goers (OR=1.20, 95% CI=0.87-1.66, p=0.273). In contrast, individuals who did not go on winter holidays were at a (weakly significant) lower risk of zoster compared with a) individuals with the lowest tertile of winter holiday exposure (**Table 6.4.3**) and b) all winter holiday goers (OR=0.73, 95% CI=0.53-1.02, p=0.053). Overall, individuals who had no (summer or winter) holidays were at similar risk of zoster compared to holiday goers (**Table 6.4.3**). The risk of zoster associated with cumulative UVR exposure from sunbathing was also not significantly associated with zoster.

Intensity of UVR exposure in the last year during non-holiday periods and from (summer or winter) holiday exposures was not significantly associated with risk of zoster (**Table 6.4.4**), neither was intensity of exposure during summer holidays and winter holidays considered as separate exposures (data not shown). Intensity of UVR exposure from sunbathing in the last year was also not significantly associated with zoster (p=0.481, data not shown). There was a weakly significant trend of increasing risk associated with increasing intermittency due to winter holidays, but no association with summer holiday intermittency. Thirty-three cases and 52 controls had experienced sunburns severe enough to cause severe erythema or blistering – sunburns were not associated with increased risk of zoster (OR=1.38; 95%CI=0.81-2.28, p=0.234). Similarly, twenty-six individuals (11 cases and 15 controls)

had sunbed and/or medical UVR exposures in the last year, which was not significantly associated with zoster risk (OR=1.49, 95% CI=0.67-3.31, p=0.106).

Wearing hats during the non-holiday warmer months was not associated with protection against zoster - 41 (16.8%) cases and 70 (14.4%) controls wore hats at least half the time (OR=1.22, 95%CI= 0.78-1.90, p=0.381). However, 37 (15.2%) cases and 102 (21.3%) controls wore protective clothing at least half the time during non-holiday periods, and this was associated with protection (OR=0.64, 95%CI=0.42-0.99, p=0.039). Amongst individuals who went on holiday, neither hat-wearing (OR=0.88, 95%CI=0.53-1.45, p=0.608) nor wearing protective clothing (OR=1.40, 95%CI=0.84-2.32, p=0.195) were significantly associated with risk of zoster.

Exposures in the month before rash onset in the case: cumulative UVR exposures from holidays and non-holidays combined and from holidays alone were not significantly associated with zoster, and there was a weak protective trend associated with cumulative UVR exposure from sunbathing (**Table 6.4.5**). However, going on holiday or on a business trip that included recreational UVR exposure in the month before rash onset was weakly associated with increased risk of zoster (OR=1.49, 95% CI=0.97-2.27, p=0.067), and there was also a weakly significant trend of increasing risk with increasing intensity of UVR holiday exposure. Intermittency of exposure due to holidays had a significant, non-linear relationship with zoster risk. Severe sunburns were not associated with zoster risk (**Table 6.4.5**), neither were medical or sunbed UVR exposures (OR=1.33, 95% CI=0.36-4.72, p=0.660).

Effect of skin type: neither a) propensity to burn on initial UVR exposure nor b) ability to tan with continued exposure were associated with risk of zoster on univariable analysis (**Table 6.4.6**). However, those with least ability to tan (individuals who freckled without tanning) were at increased risk of zoster compared with individuals who obtained a deep tan (p=0.03).

6.4.5 Multivariable analyses (Tables 6.4.7-6.4.15)

Exposures in childhood: 616 individuals (214 matched sets) had data on cumulative UVR exposures for both non-holiday and holiday periods. Amongst controls who went on holiday in childhood, there was evidence that those with high non-holiday exposure also had high holiday exposure (p<0.001). The independent effects of non-holiday and holiday UVR exposure in childhood are summarised in **Table 6.4.7**. The effect of non-holiday

exposure was slightly diminished after adjusting for holiday exposure, but remained significantly associated with risk of zoster. Other variables (LV childhood residence, skin response to UVR, current illness and wearing of hats and protective clothing) did not confound the adjusted effect estimates (data not shown), nor did ethnicity (**Table 6.4.7**, final column). In contrast to non-holiday exposure, cumulative summer holiday UVR exposure in childhood was not independently associated with zoster risk after controlling for non-holiday exposure (**Table 6.4.7**). Similar results were obtained when intensity of UVR exposure in childhood was examined, with the association with zoster remaining less strong than that of cumulative UVR dose (data not shown).

Although cumulative holiday UVR exposure was not independently associated with risk of zoster in the complete dataset, there was evidence that the adjusted effect varied with age (p for interaction = 0.018). Increasing holiday exposure was significantly associated with increased risk of zoster amongst individuals aged less than 60 years, with a quadratic pattern of association (**Table 6.4.8**). The increased risk associated with not having a holiday was also restricted to younger individuals. None of the other UVR exposure variables showed effect modification by age (p for interaction >0.2 for all)

The effect of total (non-holiday + holiday) cumulative UVR exposure in childhood could be masked by the possible increased risk associated with not going on holiday, because non-holidaymakers were more likely to have lower total UVR exposure - the lowest two quintiles of total cumulative UVR exposure contained 57.2% of the individuals who did not go on summer holidays, but only 26.8% of the individuals who had holidays. Analyses of total UVR exposure were therefore repeated amongst the matched sets (278 individuals) of holiday-goers. Effect estimates were increased for higher exposures levels in this small dataset compared to those estimated using all participants, with a weakly significant trend of increasing risk with increasing exposure (**Table 6.4.9**).

The effect of childhood intermittency of UVR exposure remained non-significant after controlling for baseline UVR exposure (data not shown). However, there was evidence that the effect of UVR intermittency due to holidays varied with level of baseline exposure (p for interaction=0.02, **Table 6.4.10**). Increasing intermittency was associated with higher risk of zoster only amongst individuals who had high daily intensity of non-holiday exposure (at least four MED/day). Again, non-holidaymakers might mask the effect of intermittency, because they were at possibly increased risk of zoster but categorised in the lowest intermittency group (because they had no holiday exposure). However, effect

estimates were very similar in the smaller dataset of holidaymakers, except for a higher magnitude of risk amongst those with the highest quintile of intermittency (data not shown).

The effect of hat-wearing in childhood did not vary according to level of either non-holiday or holiday UVR exposure (less than 5MED vs. more than 5MED, p for interaction>0.9 for both). There were not enough individuals wearing protective clothing in childhood to investigate whether the effect varied according to UVR exposure level.

Exposure in the last year: the effects of non-holiday and holiday UVR exposures remained non-significant after controlling for each other and for potential negative confounders in a multivariable model (data not shown). The weakly significant trend in increasing risk of zoster with increasing total (holiday + non-holiday) cumulative summer UVR exposure was slightly strengthened after controlling for the confounding effects of current illness, ethnicity and ability to tan, but only individuals with the highest level of exposure were at significantly increased risk of zoster (**Table 6.4.11**). The effects of cumulative UVR exposures did not vary significantly by age.

The effects of intermittency of UVR exposures in the last year remained non-significant after controlling for baseline UVR exposure and current illness, and there was no evidence that the effect of non-holiday or summer-holiday intermittency varied with baseline exposure (p for interaction >0.2 for both). Effect modification of intermittency due to winter holidays was not examined, as there was insufficient variation in winter non-holiday UVR exposure - only seven people were exposed to ≥1MED/day.

The weak protective effect associated with wearing protective clothing during non-holiday periods was not confounded by cumulative non-holiday UVR exposure, current illness, ethnicity or skin type (data not shown). The effect of wearing hats and protective clothes did not vary by level of either non-holiday or holiday UVR exposure (p for interaction>0.2 for all). Individuals with the least ability to tan (who freckled without tanning) remained at increased risk of zoster compared with individuals who obtained a deep tan after adjusting for the confounding effects of ethnicity, current illness, and total UVR exposure in the warmer months (OR=2.33, 95% CI=1.07-5.08, p=0.033). However, there was little evidence that ability to tan or propensity to burn modified the effects of any of the UVR exposures of interest (p for interaction >0.15 for all).

Exposures in the month before rash onset: the effect of total (holiday + non-holiday) cumulative UVR exposure from sunlight in the month before rash onset was strengthened after controlling for the confounding effects of current illness, ethnicity and ability to tan (**Table 6.4.11**). The adjusted estimates were more strongly associated with risk of zoster than the equivalent estimates for exposures in the last year, with higher effect estimates for all exposure levels. The effect of intensity of holiday UVR exposure in the month preceding rash onset was not confounded by non-holiday UVR exposure, ethnicity, current illness or skin type (data not shown).

There was evidence that age modified the effect of total UVR exposure in the month before rash onset (p for interaction=0.015), with a more strongly significant risk of zoster in individuals aged less than 60 years, although the pattern of risk was neither linear nor quadratic in either age group (**Table 6.4.12**). The effects of other UVR exposure variables did not vary with age. Numbers were insufficient to investigate whether the effect of wearing of hats and protective clothing varied according to level of holiday UVR exposure.

There was also evidence that the effect of total holiday UVR exposure in the month before rash onset was modified by propensity to burn (p for interaction=0.046), although again the risks had no clear pattern (**Table 6.4.13**). Amongst individuals who tanned on initial UVR exposure, there was a trend towards increasing risk of zoster with increasing holiday UVR exposure compared to those who did not go on holiday, but effect estimates did not reach statistical significance. Amongst individuals who burned or peeled on initial exposure, the risk of zoster was significantly higher amongst individuals who had relatively low holiday exposures, but not amongst individuals with high holiday UVR exposures. However, only two cases and eight controls were in the top category of exposure.

Exposure to UVR is known to increase the risk of immediate reactivation of latent herpes simplex virus (HSV) infection, the clinical presentation of which can occasionally be mistaken for zoster.[44,234,348,357] The effect of using a 'probable' zoster case definition with imperfect specificity was examined by restricting analyses of UVR exposures in the month before rash onset to the small datasets of confirmed cases and their matched controls. Power was reduced due to the small numbers, but effect estimates for total (holiday + non-holiday) UVR exposure amongst confirmed cases and matched controls were of greater magnitude than those for the whole dataset, and this effect persisted when data were further restricted to younger individuals (a group at higher risk of HSV reactivation compared to older individuals) (**Table 6.4.14**).

The effects of different UVR exposures for the three time periods are summarised in **Table 6.4.15**.

6.4.5 Discussion

A strong effect of cumulative UVR exposure in the warmer months of childhood was identified, with a quadratic pattern of increased risk of zoster. This is similar to the pattern of association between early life UVR exposure and subsequent risk of basal cell carcinoma, reported by Kricker *et al.*[287] Individuals' 'usual' (non-holiday) childhood UVR exposure was associated with increased risk of zoster amongst participants of all ages, but high holiday UVR exposure in childhood and a lack of childhood holidays were associated with increased risk only amongst younger individuals. Participants who had no childhood holidays also tended to have high non-holiday UVR exposure. However, increased zoster risk amongst those without holidays remained after controlling for non-holiday UVR, indicating that some of the risk resulted from other factors. Total (non-holiday + holiday) cumulative childhood UVR exposure was not significantly associated with zoster risk, but this may be partly due to inclusion of the majority of non-holidaymakers (a group at possibly intermediate risk of zoster) in the lower quintiles of total exposure.

Recent cumulative UVR exposure in adulthood was less strongly associated with risk of zoster. Unlike childhood exposure, there was some evidence that total (non-holiday + holiday) cumulative exposure in the last year was associated with increased zoster risk, but no association with either non-holiday or holiday exposure considered separately. In general, quintiles of childhood exposure for these variables had much higher values compared to the equivalent quintiles for recent exposure. Also, the effect of not taking summer holidays in the last year was less marked than the effect of no holidays in childhood, and so may have had less influence on the risk associated with the lowest quintiles of total UVR exposure. There was limited power to investigate the effects of cumulative UVR exposures in the month before rash onset, as the data included many individuals with similar exposure levels - for example, where zoster had occurred in the case during the winter months. Nevertheless, adjusted effect estimates of total cumulative exposure in the last month were slightly more strongly associated with risk of zoster compared with exposure in the last year, suggesting that recent exposure may affect zoster risk.

In general, intensity of UVR exposure at each time period had a weaker association with zoster compared to cumulative exposure, except for intensity of holiday UVR exposure shortly before rash onset. Estimates of exposure intensity used the maximum UVR dose received between

9am and 5pm on any one day, without taking into account how long it took to receive this dose. There may have been considerable variation between individuals in the hourly intensity of exposure *within* a day – for example, a daily dose of 4 MED may have been experienced over eight hours by one individual but in a single hour by another, depending on their location of residence or holiday destination. This may explain some of the difference between younger and older individuals in the effect of cumulative holiday exposure in childhood. Younger individuals went to holiday destinations with significantly higher daily ambient UVR compared to older individuals, and spent fewer hours outdoors. So, younger individuals may have experienced the same daily UVR dose on holiday as older individuals but received a more intense dose within that time, and this may have increased their risk of immunosuppression.

Other patterns of UVR exposure showed less strong associations with zoster. High intermittency of exposure in childhood was not associated with increased risk overall, and results of stratified analyses indicated that high childhood intermittency due to holidays was associated with increased zoster risk only amongst individuals who also had high non-holiday exposure. This suggests that it is total UVR dose that increases risk, and not simply a wide differential in exposure. Winter non-holiday exposure was low in almost all individuals, and the weak effect of recent winter holiday intermittency may simply have represented the effect of acute exposure amongst individuals who were not acclimatised to the sun. However, neither cumulative winter holiday exposure nor intensity of exposure were significantly associated with risk of zoster.

There was some evidence that 'frecklers' (individuals with poor ability to tan) were at increased risk of zoster. Recent UVR exposure also had a greater effect amongst those with a propensity to burn, but there was limited power to examine this because few individuals who burned easily had high levels of UVR exposure. Effect modification by skin type is consistent with the hypothesis that individuals with poor skin tolerance to the sun might be particularly susceptible to UVR-mediated immune suppression.

Hats were not found to be protective against the effect of UVR on zoster risk, nor was clothing in most situations. The protection from hats may be limited as they only protect the head area from the sun, and the protective effect of clothing can be diminished by fabric colour, content, age and hydration.[358,359] In addition, individuals who reported wearing protective clothing on holiday or during leisure activities may have subsequently increased their UVR exposure, believing themselves to be protected against the sun. Only individuals who wore protective clothing for most of the time during non-holiday periods were at possible lower risk of zoster.

These individuals may have been 'sun-avoiders', who wore appropriate protective clothing and actively limited their exposure to intense UVR. The lack of association of zoster risk with use of sunbeds, medical exposures and sunburns may have been due to small numbers of exposed individuals.

Why might childhood UVR exposure increase the risk of zoster in adulthood? One possibility is that high UVR exposure in early childhood 'programmes' the immune system so that it responds less robustly to subsequent challenges. This might be particularly marked where exposures are intense (such as on holiday), leading to young age at zoster. Alternatively, variation in UVR exposures in childhood may represent a broad range of behavioural activity, some of which might be associated with immune programming – for example, childhood infections, vaccination status or endotoxin exposure.[360-364] Information was not collected on these childhood factors, or on proxy factors (such as childhood socio-economic status), and it was not possible to assess their influence. Recent UVR exposure might affect the risk of zosfer by direct immunosuppression, facilitating reactivation of latent VZV infection. It was hypothesised that this effect might be more marked in older individuals, due to less robust functioning of the aging immune system. However, there was no evidence that recent UVR exposures had a greater effect in older individuals in this study.

The quadratic pattern of childhood UVR exposure risk is intriguing. In the case of basal cell carcinoma, UVR-induced genetic mutations in skin cells increase with increasing UVR exposure.[365,366] Kricker *et* al suggested that the cells may become non-viable at very high UVR exposure levels (due to accumulated mutations), and are destroyed before they can go on to become cancerous – therefore individuals with very high UVR exposure may be at lower risk of skin cancer compared to those with intermediate exposure.[287] As outlined in Chapter 2, UVR may cause immunosuppression by damaging keratinocyte DNA, and the altered keratinocytes produce cytokines that preferentially activate TH_2-type helper cells. Perhaps at very high UVR exposure levels the DNA damage is so great that the keratinocytes are destroyed, and so cytokine production causing the shift to TH_2-type helper cells (which may result in immune programming) is switched off.

One potential limitation of these analyses is the possible misclassification of HSV infection as zoster amongst 'probable' cases, resulting in an erroneous association with recent UVR exposure. Subgroup analyses of confirmed cases indicates that misdiagnosis of zoster was unlikely to have contributed greatly to the weak effect associated with recent exposure. Other limitations to these data include:

1. *Misclassification of ambient UVR levels at residences and holiday destinations:* this was likely to be considerable. Average UVR levels and cloud cover were used for the half-year periods comprising the warmer and the cooler months. No account was taken of altitude or surface reflectance, which may have a pronounced effect on the amount of ambient UVR.[172] This non-differential misclassification may have led to underestimation of the effects of UVR exposures.

2. *Misclassification of personal UVR exposure:* in addition to misclassification of ambient UVR exposure, participants probably misreported how long they spent outdoors. Also, individuals were asked only about time spent outdoors between 9am-5pm, with no supplementary questions asked about exposure between 11am-3pm (the time of maximum UVR dosage). Misreporting is likely to have been similar in cases and controls, and so may have contributed to underestimation of UVR effects. It is interesting that childhood UVR exposure was strongly associated with zoster risk despite possibly high levels of misreporting by participants. Perhaps this reflects the advantage of using residence calendars, and prompting individuals with standardised questions to aid memory of childhood events.

3. *Health effects:* this may have obscured the relationship between recent UVR exposure and zoster. Individuals who feel unwell may spend more time indoors, and may not go on holiday. If these individuals have lower functional immunity (resulting in increased susceptibility to zoster), associations between zoster and UVR may be negatively confounded. This may explain the weak protective effect of sunbathing in the month before rash onset. Controlling for current illness may have not have removed this problem if individuals with generalised malaise were not formally diagnosed as being ill. In addition, some cases may have felt unwell due to prodromal zoster, and reduced their UVR exposure shortly before rash onset.

4. *Effect of past UVR exposure on recent exposure:* if long-term UVR exposure increases the risk of zoster, it may confound the effect of recent exposures under some circumstances. For example, individuals with high UVR exposure in the last twenty years may have developed UVR-related skin problems or have suffered severe sunburns, and then modified their sun-seeking behaviour.

5. *Other confounding:* other risk factors for zoster may explain some of the associations found in these analyses. For example, stress may have confounded the weak effect of recent winter holiday exposure. Potential confounders were explored in the combined model (Section 6.8, below).

6. *Delay between interviewing cases & controls:* controls were interviewed after cases, but differences in recall of UVR exposure in the last year are likely to have been non-systematic. For example, at interview controls had sometimes been on holiday more recently compared to their matched cases, and sometimes less recently. Answers may have been influenced by recent weather changes – time spent outdoors in the warmer months can vary considerably according to the weather, and reporting may have reflected most recent exposure rather than average exposure. Again, this may have lead to non-differential misclassification of exposure. The situation for UVR exposure in the month before rash onset in the case was somewhat different, where the period of interest was immediately before interview for cases but sometimes much earlier for controls. As with dietary intake (discussed earlier in this Chapter) individuals were asked about usual UVR exposure in the last year without asking them about a specific time period, and exposure in the month before rash onset was calculated subsequently. Nevertheless, delays in interviewing controls could have led to differential misclassification of UVR exposure.

The results of these analyses suggest that childhood (and possibly recent) UVR exposure may have an important effect on susceptibility to zoster. Extensive data were collected at interview on lifetime exposure to UVR. This will enable future analyses of the effects of long-term UVR exposure on zoster risk, including potential confounding or effect modification of recent UVR exposure by past exposure.

6.5 STRESS AND ILLNESS

6.5.1 Specific hypotheses

The primary hypotheses were that:

1. Stressful events in the last 12 months and/or in the two months before rash onset increase the risk of zoster.
2. Illnesses in the last 12 months and/or in the two months before rash onset that affect cell-mediated immunity increase the risk of zoster.

Investigation of the two periods allowed assessment of the effects of both longer-term and acute stress and illness. Two months was chosen to investigate the effects of acute stress,

because (as outlined in Chapter 2) studies of bereaved spouses have shown that lymphocyte function is impaired in the first two months after the acute stress of bereavement.[136,137]

6.5.2 Data conversion and categorisation

Individuals were prompted at interview about 12 types of stressful events (see Chapter 3, Section 3.9.1 and **Appendix 7**), and were also asked an open question about whether they had experienced any other stressful event in the last year. Responses to the open question included both stressful events (such as accidents or trouble with the police) and stressful feelings (such as feelings of isolation from the family or concerns about the participant's own health). Therefore, the data comprised a mixture of a) stressful events that were reported in response to the 12 standardised questions asked of all participants, and b) stressful events and feelings that were volunteered by some participants in response to the open question. The following three categories were generated:

1. *Prompted events:* these were the 12 specific types of stressful event that were routinely asked of all participants. Multiple responses to each question (for example, if two close family members had been seriously ill in the last year) were counted as separate events.
2. *Unprompted events:* these were events reported in response to the open-ended question
3. *Unprompted feelings:* these were feelings reported in response to the open-ended question

Individuals' positive responses were summed, to obtain the total number of stressful events/feelings overall, and the number in each of the three categories. For events that occurred over time (such as family illness), information was collected on when the event began and ended. This allowed categorisation of stressful events in the two months before rash onset into *incident* events (those starting less than two months before rash onset) and *prevalent* events (those starting earlier than two months before rash onset but extending into the two month period).

Information was sought at interview about major medical conditions and treatments in the last year. Individuals were excluded from the study if they had an underlying condition that was associated with depressed cell-mediated immunity, as listed in Chapter 3 (Section 3.7.3). However, remaining illnesses were considered relevant to the present analyses for two reasons. Firstly, individuals may experience being ill as a stressful event. Secondly, a few individuals had conditions that potentially could affect immune functioning (listed below). Subcategories of illness investigated as potential risk factors for zoster included:

1. Medical conditions or treatments possibly associated with impaired immune functioning – insulin-dependent diabetes mellitus, Down's syndrome, chronic fatigue syndrome, chronic renal failure, pregnancy and low-dose oral steroids (taken by one 91-year old control for whom a replacement could not be found).
2. Illnesses or treatments possibly associated with altered micronutrient availability or requirement (listed earlier in this Chapter, Section 6.3.2).
3. Major infections
4. Hospitalisations in the last six months
5. Surgical procedures in the last six months, subdivided into minor invasive procedures and major procedures necessitating general anaesthetic.
6. Psychiatric illness, and/or prescriptions for anxiolytic or antidepressant medication

6.5.3 Analytical strategy

Univariable analyses were undertaken of stressful events/feelings in the last 12 months and in the two months before rash onset. Analyses included:

1. The effect of the total number of events/feelings (prompted and unprompted combined), and of each of the three stress variables (prompted events, unprompted events and unprompted feelings).
2. The effect of each stressful factor (the effects of death of a spouse, death of a close relative, etc), considered singly or in groups of related factors (such as the three 'death' stress variables) where numbers were small.
3. The effects of recent illnesses.

Multivariable models were set up for each period - the last 12 months and the two months before rash. The models were built as follows:

1. Stress events/feelings were added first to the model. Two alternative models were used, a) using the total number of (prompted+unprompted) events/feelings, and b) adding prompted stress events, unprompted events and unprompted feelings separately, as ordered categorical variables if there were sufficient numbers. The second model allowed investigation of the independent effects of prompted and unprompted events/feelings.

2. Illness variables were added to the model, and retained if they were independently associated with risk of zoster or if they confounded any of the stress variables. Again, two alternative models were used, a) using a variable encompassing any major medical or surgical condition, and b) using variables representing specific illnesses, listed above. In the second model, the variable representing illness in general was added after adding specific groups of illnesses, to estimate whether illnesses not included in the specific variables also had a confounding or independent effect on risk of zoster.

3. Alcohol consumption and smoking may confound or lie on the causal pathway between stress and risk of zoster, because they are commonly associated with stress events and may also affect cell-mediated immune functioning.[311-314] These two variables were therefore added to the model, and retained if they altered the effect estimates of any of the stress or illness variables. The potential confounding effects of socio-economic status variables (house tenure and car ownership) and ethnicity were also examined.

4. Effect modification of stress variables by a) age and b) illness was investigated. Interactions between incident and prevalent stress events in the two months before rash onset were also examined, to see whether the effect of acute stress was exacerbated by chronic stress.

6.5.3 Univariable analyses

The effects of stressful events or feelings in the 12 months before interview are summarised in **Table 6.5.1**. Increasing numbers of total (prompted+unprompted) events/feelings were associated with a graded increasing risk of zoster - individuals reporting five or more events/feelings were at more than three times the risk of zoster compared to individuals with no reported events. When individual stress variables were examined, unprompted events and unprompted feelings both had dose-response effects, although the latter was only weakly statistically significant. The effect of prompted stress events had a weaker, less graded association with risk of zoster. However, individuals reporting five or more prompted events were at more than double the risk of zoster compared to individuals reporting no events. Individual events/feelings that were most significantly associated with zoster included serious illness amongst family members, organising weddings or parties and a feeling of continuing bereavement.

The univariable effects of *incident* stressful events or feelings in the two months before rash are summarised in **Table 6.5.2**. Again, there was a significant graded association between risk of zoster and total number of (promped+unprompted) events/feelings. Both incident

prompted events and unprompted events were significantly associated with zoster risk, with exposed individuals at more than twice and more than four times the risk of zoster respectively. Few individuals reported stressful feelings that started in the two months before rash, and these were not significantly associated with zoster risk. Individual events/feelings that were most significantly associated with zoster included death of a spouse, close relative or close friend, difficulties with work, accidents, robberies or assaults, and moving house.

The effects of *prevalent* stressful events in the two months before rash onset were in general less strongly associated with risk of zoster compared to incident events (**Table 6.5.3**). Prevalent stressful feelings were weakly associated with increased risk of zoster, of which the strongest association was that of feelings of continuing bereavement.

The univariable effects of recent illness on risk of zoster are summarised in **Table 6.5.4**. Individuals who reported any major medical or surgical condition in the last year were at nearly twice the risk of zoster compared to those who did not report major illness. Most of the specific conditions examined were not significantly associated with zoster risk. However, as shown earlier in this Chapter, illnesses or treatments leading to altered micronutrient availability or requirement were associated with more than a three-fold increased risk of zoster. Serious infections in the last six months were associated with more than twice the risk of zoster, and serious infections in the two months before rash onset were weakly associated with increased risk. Hospitalisations were associated with protection against zoster, but this did not reach statistical significance.

6.5.4 Multivariable analyses

Stress in the last 12 months: the effect estimates for the total number of stressful events/feelings in the last year were little changed after adjustment for either recent medical conditions or specific illnesses (**Table 6.5.5**). Similarly, the three individual stress variables were not confounded by each other or by illnesses. The effect of having any medical condition was slightly diminished after controlling for the effect of stress, but remained significantly associated with zoster risk, even after controlling for specific illnesses variables. Of these illnesses, the effects of serious infections and conditions associated with altered micronutrient availability were diminished in the multivariable model, but remained significantly or weakly significantly associated with zoster risk. The protective effect of hospitalisations increased, so that individuals experiencing hospitalisation in the last six months were at approximately two fifths the risk of zoster compared to non-hospitalised

individuals. None of the stress or illness variables were confounded by socio-economic status, ethnicity, alcohol consumption or smoking.

There was evidence that the effects of a) all stress events/feelings and b) prompted stress events varied with age (p for interaction= 0.002 and 0.035 respectively). For each variable, stress was associated with a non-linear protective effect in individuals less than 60 years old, but with a non-linear increased risk amongst older individuals (**Table 6.5.6**). There was no evidence of interaction between stress variables, or between stress and most illness variables. However, there was an interaction between stressful feelings and hospitalisations (p for interaction=0.009). Hospitalisation was associated with strong protection against zoster amongst those who did not report stressful feelings in the last year (OR=0.08, 95%CI=0.01-0.61, p=0.015), but was not associated with risk of zoster amongst those who reported stressful feelings (OR=0.97, 95%CI=0.35-2.67, p=0.956).

Stress in the two months before rash onset: the effect of the total number of incident stressful events/feelings in the two months before rash was little changed after adjusting for the effects of prevalent stress and any medical conditions (**Table 6.5.7**). However, the total number of prevalent stresses was only weakly associated with zoster risk in the multivariable model. Of the individual stress variables, incident prompted and unprompted stress events and prevalent unprompted stressful feelings remained significantly associated with risk of zoster after controlling for one another and for the effects of recent illness (**Table 6.5.7**). Individuals with medical conditions remained at more than one and a half times the risk of zoster compared to individuals with no illness. When specific illnesses were considered, only conditions associated with altered micronutrient requirement/availability were independently associated with zoster after controlling for recent stress (OR=2.60, 95%CI=1.04-6.50, p=0.037). Other illness and stress variables did not confound the effect estimates for stress variables, nor did socio-economic status, ethnicity, alcohol intake or smoking. There was no evidence of interactions between the stress or illness variables in the model. Unlike stress in the last year, there was also no evidence of effect modification by age.

6.5.5 Discussion

Individuals who reported a high number of stressful events and/or feelings in the twelve months before interview were at increased risk of zoster. Of these, events or feelings that were unprompted (volunteered by the participant) were strongly related to increased risk, but prompted events (responses to standardised questions about major life events) were less

strongly associated with zoster risk. This is similar to the findings of Schmader *et al*, where elderly cases of zoster and matched controls had a similar number of major life events in the year before zoster onset.[153] However, in the present study the effect of prompted events in the last year (and the effect of all events/feelings combined) varied with age, with some evidence of decreased risk amongst younger individuals but increased risk amongst older individuals. As mentioned in Chapter 2, the effect of stress on the immune system may depend on individuals' defence and coping mechanisms, and can result in heightened immune responses.[143-146] It is possible that stress affects the ageing immune system differently to the more robust immune system of younger individuals, or that older individuals are more likely to experience major life events as being stressful.

In contrast to events in the last year, individuals reporting prompted stressful events that first occurred in the two months before rash onset in the case were at twice the risk of zoster, and this effect was not explained either by recent illness or by other stressful events. Schleifer *et al* and Bartrop *et al* showed that lymphocyte function was diminished in the first two months following acute stressful events.[136,137] Schmader *et al* found that although cases did not have significantly higher numbers of stressful events in the two months before rash, the number of self-rated 'negative' events were associated with zoster risk.[153] In general, recent prevalent stress was less strongly associated with risk of zoster compared to recent incident stress, and did not exacerbate the effects of acute stress. Schleiffer *et al* demonstrated that suppressed immune responses in the two months after bereavement were followed by intermediate levels of immune responsiveness in the next 4-14 months.[137] Perhaps the lower magnitude of risk associated with prevalent stress reflects adaptation to the effects of stress over time.

Conditions that affected micronutrient availability or requirement, and serious infections in the last six months were independently associated with risk of zoster. Both of these sets of conditions may affect cell-mediated immunity, and thus increase risk of zoster. However, individuals with other current medical conditions (for example cardiovascular disease) remained at increased risk of zoster after adjusting for specific illnesses that might affect immune function. This is consistent with the findings of Schmader *et al* that elderly individuals who self-rated their health as 'excellent' were at half the subsequent risk of zoster compared to other individuals.[80] The increased risk associated with general ill-health may be mediated by unreported stress generated in response to being unwell, or other effects of poor health on immune functioning. Hospitalisations in the last six months were protective against zoster, but only amongst those who did not report stressful feelings. Eight of the nine (89%) cases that were hospitalised reported stressful feelings, mostly

relating to concerns about their own health. In contrast, only ten (34%) of the 29 hospitalised controls reported stressful feelings, and many controls attended hospital for scheduled operations. Therefore, the apparent protective effect of hospitalisation may be due to differences in cases' and controls' underlying medical history and experience of hospitalisation.

How should the three types of stress variable (prompted events, unprompted events, and unprompted feelings) be interpreted? Prompted events represent major life events likely to be associated with stress. Questions about these events were adapted from instruments used in previous studies.[80,153,315] Unprompted events reported by interviewees included a number of major events such as being assaulted or court cases. This suggests that the variable representing the total number of (prompted and unprompted) stressful events/feelings may reflect differences in major stressful events experienced by the study population.

However, use of an open question about stress increases the possibility of recall bias, particularly in the reporting of less major events and stressful feelings. Recall bias may explain much of the difference in magnitude of effect between unprompted and prompted stress events in the last year. Even some of the prompted events (for example, trouble with neighbours or difficulties at work) may be prone to recall bias. Differences in reporting of less major stressful events in cases and controls in the two months before rash onset may also have been exacerbated by the delay in interviewing some of the controls relative to their matched cases.

The results may also have been affected by participation bias. It is likely that individuals suffering acute stress or serious illness might be less willing to take part in the study, and this might be particularly true for controls. The reasons for non-participation were unknown for many individuals, but refusals included: severe depression (one case and one potential control), recent bereavement (two controls), ongoing bereavement (two cases and one control), spousal or family illness (six controls), hospitalisation (three controls), other ill health (one case and eight controls), and a recent accident (one control). Although overall levels of participation were high, participation bias may have contributed to some of the effects reported.

Despite these limitations, there was evidence that stressful events increased the risk of zoster. The difference in the magnitude of effects associated with unprompted and prompted stress is an indicator of the strength of recall bias. However, the difference of the effect of prompted events amongst younger and older individuals suggests that there may be

a real effect of stress on risk of zoster. The confounding effect of stress on other risk factors for zoster is examined later in this Chapter.

6.6 PHYSICAL TRAUMA

6.6.1 Specific hypothesis

The primary hypothesis was that mechanical trauma in the six months before interview and/or in the month before rash onset increases the risk of zoster. Trauma in the month before onset was chosen because there have been case reports of zoster occurring within a month of specific trauma.[34,156,367,368] It was also hypothesised that the risk of zoster would be greater when the site of the trauma was the same as the site of subsequent rash.

6.6.2 Data categorisation

Participants were asked about physical injuries (for example, falls or knocks that were severe enough to cause bruising) in the last six months, with details about the site of the injury and when it occurred. Information about trauma due to invasive surgery was acquired from the medical history. These data were combined to obtain a history of trauma at the same site as subsequent rash in the case, and a history of trauma at different sites.

6.6.3 Analytical strategy

Univariable analyses were undertaken of physical trauma in the last six months and in the month before rash onset in the case. Trauma to the same site as subsequent rash and trauma to different sites were both examined, to investigate the specific and general effects of trauma on risk of zoster. Multivariable analysis included adjustment for potential confounders of the effect of trauma, including current illness and alcohol consumption. Effect modification by age was also investigated.

6.6.4 Univariable analyses

The univariable effects of physical trauma on risk of zoster are summarised in **Table 6.6.1**. Trauma in the last six months to any site was not associated with risk of zoster, but individuals who experienced trauma in the month before rash onset were at more that twice the risk of zoster. Trauma at the same site as subsequent rash in the case in the last six

months was associated with a ten-fold increased risk of zoster, and trauma in the month before rash was associated with a nineteen-fold increased risk (although 95% confidence estimates for both estimates were wide). However, trauma at a different site to the rash was not associated with a significantly increased risk for either time period.

6.6.5 Multivariable analyses

The effect estimates of physical trauma to any site and trauma to the same site as subsequent rash were little affected after adjustment for current illness (**Table 6.6.1**, column 5). Alcohol did not confound any of the effects of interest, and the two site-specific trauma variables (trauma to the same site and trauma to a different site) did not confound one another (data not shown). There was no evidence that age modified the effect of trauma in the last six months (p for interaction>0.2 for all three trauma variables). There were insufficient numbers to ascertain whether age modified the effect of trauma to the same site in the month before rash onset. However, there was evidence that the effect of trauma to any site and to a different site in the month before rash varied with age (p for interaction=0.01 and 0.033 respectively). Amongst individuals aged less than 60 years, nether trauma variable was significantly associated with zoster after adjusting for current illness (**Table 6.6.2**). In contrast, older individuals who experienced trauma to any site were at more than six times the risk of zoster, and those who experienced trauma to a different site to subsequent rash were at more than four times the risk of zoster compared to individuals with no trauma.

6.6.6 Discussion

Physical trauma is associated with an increased risk of zoster, and this effect is stronger for trauma sustained shortly before rash onset. This is consistent with the findings amongst the case-series reported by Juel-Jenson and other case reports that could not estimate relative risk because controls were not employed to ascertain history of trauma in the population.[34,156,367,368] In this study, only trauma at the site of subsequent rash was associated with zoster risk when all individuals were considered. This suggests that mechanical trauma may trigger reactivation of latent virus in the dorsal root ganglion of the nerve supplying the affected dermatome. However, the risk of zoster associated with trauma to other sites was also increased amongst older individuals. This might occur because the psychological stress following falls or accidents may affect immune functioning, particularly amongst elderly individuals with ageing immune systems. Alternatively, physical trauma may not be associated with risk of zoster, but may appear to

be so because it is a consequence of general poor health not fully captured by reported current illness.

The major limitation of these analyses is the possibility of recall bias, particularly for trauma to the site affected by zoster. This may have been exacerbated by the delay in interviewing some controls, who may have forgotten less serious trauma. As only a few individuals experienced trauma to the affected site and because there is a strong possibility of recall bias, only tentative inferences can be made from these analyses.

6.7 OTHER CONFOUNDERS

Independent risk factors for zoster within each of the five main models (child/varicella contacts, ethnicity/country of birth, micronutrient intake, ultraviolet radiation exposure and stress/illness) also potentially confounded one another. This was examined in the final model, described next in this Chapter. However, a few variables were considered purely as confounders, and were not investigated as independent risk factors within the five models. These included:

a) Smoking

b) Alcohol intake

c) Socio-economic status - housing tenure and car ownership

The potentially confounding effects of these variables in each of the main models have already been described. Their univariable effects on risk of zoster are reported here, for completeness.

6.7.1 Data categorisation

Individuals were categorised as non-smokers, ex-smokers and current smokers, and the number of cigarettes smoked per day in the last year or in the two months before rash onset were divided into 'none', 1-9, 10-19 and ≥20. Daily alcohol intake in the last year and/or in the two months before rash onset was calculated in the same way as micronutrient intake (described in Chapter 4), using information on frequency of consumption, 'portion size' and the alcohol content of each alcoholic drink consumed. Intakes were then subdivided into 'none' and into quartiles of exposure, based on the distribution amongst controls. Socioeconomic variables were categorised as on the questionnaire (see **Appendix 7**).

6.7.2 Univariable analyses

The effects of the potential confounders on risk of zoster are summarised in **Table 6.7.1**. None of the variables were associated with zoster, either as categorical or as linear variables. The effects of alcohol and cigarette consumption in the two months before rash onset were very similar to those of consumption in the last year (data not shown).

6.7.3 Discussion

None of the potential confounders were significant risk factors for zoster on univariable analysis. This is consistent with their lack of confounding for most of the other variables in the five models. The effects of cigarette and alcohol consumption may have been underestimated, due to non-differential misclassification of exposure. However, smoking status slightly confounded the effect of fruit consumption, and was independently associated with risk of zoster in the final model (described below).

6.8 COMBINED MODEL

The multivariable sub-models described above were used to assess the independent effects of related variables. However, these analyses did not take into account that variables from different sub-models might also confound one another. Therefore, a combined model was set up, to investigate the independent effects of all the variables of interest.

6.8.1 Analytical strategy

Variables from the five main multivariable sub-models were combined to obtain a single model, using a dataset of 217 cases and 411 controls with no missing data. Variables were eligible for inclusion in the combined model if they were significantly associated with zoster to the level of $p \leq 0.1$ in their sub-models, and/or if they were a confounder of any of the other significant variables.

Sub-model analyses were repeated for the reduced (n=628) dataset, to re-establish baseline effect estimates within the sub-model. The five sub-models were then combined. Confounding was assessed by comparing sub-model effect estimates with those obtained in

the combined model. Variables were dropped from the combined model if they were not significantly associated with zoster ($p>0.1$) and did not confound the other variables.

Some sub-model analyses used more than one model or alternative variables – for example, the 'child contacts' analysis had one model for child contacts in the last 10 years and a second model for contacts in the last year, and the 'food model' used fruit intake and vegetable intake either as two separate variables or as a combined single variable. Where alternative variables or more than one version of the sub-model existed, the following strategy was used:

1. The variable or group of variables (sub-model) that were most strongly associated with zoster risk were first added to the combined model, to ascertain the independent and confounding effects of these variables.
2. These variables were replaced by variables from the alternative sub-model, to determine the independent effects of the alternative variables in the combined model.

Some variables were excluded after univariable or sub-model analysis, because they were not associated with risk of zoster ($p>0.2$ for univariable analyses, $p>0.1$ for sub-model analyses). Some of these excluded variables were reassessed in the combined model, to check whether their effects had been masked by negative confounding. Variables were selected for reassessment by a) using a more generous cut-off for association on univariable analysis ($p<0.4$), and b) including variables with strong hypothesised links with risk of zoster. The latter variables included occupational contacts with well children, dietary micronutrient intake, holiday UVR exposure in childhood, skin type (ability to tan and propensity to burn), and serious infections in the last six months.

The effect of some variables was restricted to either older or younger individuals in sub-model analysis. These age-specific effects were re-examined in the combined model, to see whether they persisted after controlling for a wider range of potential confounders. Due to small numbers, other variables were only retained in the age-specific models if they confounded the effect estimates of interest.

6.8.2 Findings

The variables with the most significant effects in sub-model reanalyses (using the reduced dataset) are listed in **Table 6.8.1** (3rd column). In general, effect estimates were similar to the estimates obtained from the original (larger) datasets described earlier in this Chapter.

Further details of revised subgroup analyses and estimates obtained from the combined model are described below and summarised in **Table 6.8.1** (main variables) and **Table 6.8.2** (alternative variables).

Child contact variables: in revised sub-group analyses, the effects of the intermediate child contact variables (contacts with many children) were weakened after adding varicella contacts to the sub-model (data not shown). In the combined model, child and varicella contacts in the last 10 years remained significantly protective against zoster (**Table 6.8.1**, final column). Occupational contact with multiple ill children was associated with increased protection after adjusting for the effects of variables from other sub-models – the major negative confounders were unprompted stress in the two months before rash onset and non-holiday UVR exposure in childhood. Occupational contact with many well children (e.g. teaching) remained unassociated with risk of zoster in the combined model (OR=1.31, 95%CI=0.65-2.63; p=0.452).

Child and varicella contacts in the last year were considered in the combined model as alternative variables to contacts in the last ten years. Estimates for these variables were little altered in the combined model, except that the magnitude of increased risk associated with a single varicella contact was increased (**Table 6.8.2**).

Food and micronutrient intake: individual micronutrient intakes were not significantly associated with risk of zoster in the combined model - p values ranged from 0.14-0.94 (data not shown), consistent with original univariable analyses. In addition, (fresh or frozen) vegetable intake in the last year was not significantly associated with zoster in the final model and did not confound the effects of other variables (data not shown). Therefore, vegetable intake was dropped from analyses. Low fruit intake in the last year was associated with a higher risk of zoster in the combined model compared to the revised sub-model (**Table 6.8.1**). This was mainly because many individuals with the lowest fruit intake had a high number of contacts with specific children outside the household, and had low non-holiday UVR in childhood. Combined fresh fruit and vegetable intake was assessed as an alternative variable to fresh fruit intake in the final model. This was associated with a graded increased risk of zoster in the revised sub-model, and effect estimates were little altered in the combined model (**Table 6.8.2**).

In the original sub-model, *combined* micronutrient intake (measured as a micronutrient score) was not associated with risk of zoster amongst all individuals, but low micronutrient scores were associated with increased zoster risk amongst older individuals (Section 6.3.5,

above). In the combined model, micronutrient intake was again not significantly associated with risk of zoster overall (p=0.981), but the increased risk amongst older individuals persisted after controlling for the confounding effects of social contacts with children (those not living in the household and children in groups), childhood non-holiday UVR exposure, current illness, and incident prompted and unprompted stress events (**Table 6.8.3**). Effect estimates were higher than those in the original sub-model, but confidence intervals for some of the effect estimates were very wide.

Ultraviolet radiation (UVR) exposure: weekly non-holiday UVR exposure in the warmer months of childhood was more strongly associated with risk of zoster in the combined model compared to the sub-model, and retained a quadratic pattern of dose-response (**Table 6.8.1**). Total holiday UVR in childhood was dropped from analyses, because it was not significantly associated with zoster in the combined model and did not confound any of the effect estimates of interest. However, in the original sub-model total childhood summer holiday UVR exposure was associated with increased risk of zoster amongst younger individuals (aged <60 years). In revised analyses, the effect of childhood holiday UVR exposure was confounded by non-holiday exposure, ethnicity, time since chickenpox and contacts with varicella cases in the last ten years. The adjusted effect estimates retained a quadratic pattern of increased risk amongst those who went on holiday (n=187), although this could not be tested formally due to small numbers (**Table 6.8.3**).

Total UVR exposure in the month before rash onset in the case was considered as an alternative variable to UVR exposure in childhood. As with the original analyses, increasing UVR exposure was associated with a non-linear increasing risk of zoster (**Table 6.8.2**). The effect estimates for the highest levels of exposure were greater than estimates in the sub-model, due to negative confounding by a number of other variables. Ability to tan and propensity to burn were not associated with risk of zoster in the combined model (p=0.462 and 0.829, data not shown)

Stress and illness: the effect estimates for incident prompted stress events and for prevalent stressful feelings in the two months before rash increased slightly in the combined model (**Table 6.8.1**). The effect estimate for incident unprompted stress events showed the greatest increase - the major negative confounders were occupational and social child contacts.

Stress events in the last year were examined as an alternative to events in the two months before rash (**Table 6.8.2**). Individuals with five or more prompted stress events in the last 12 months were at nearly seven times the risk of zoster compared to those with no events

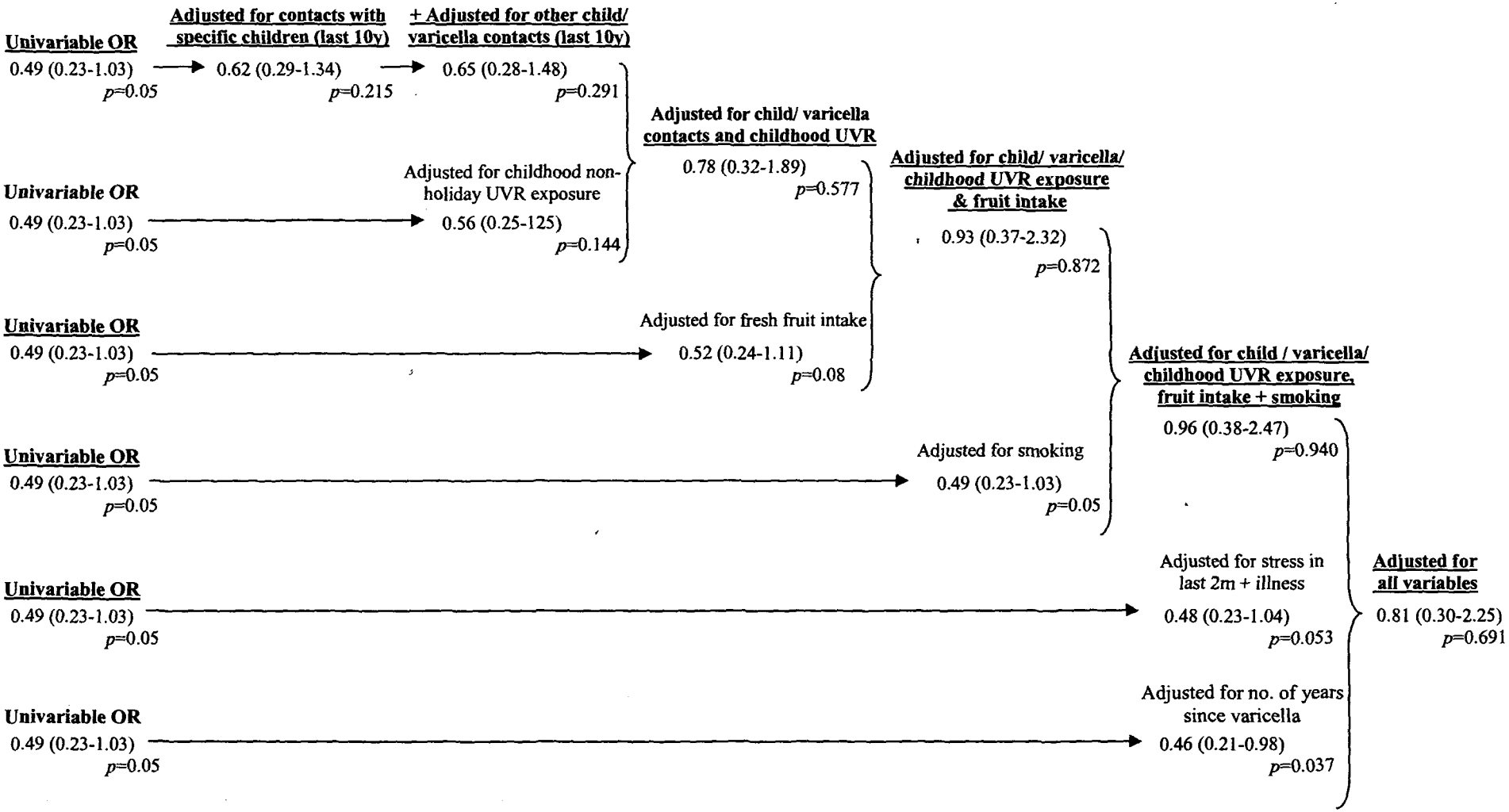
after controlling for the effects of other risk factors for zoster. In contrast, unprompted stress events had a less graded association with risk of zoster in the combined model, and the effect of stressful feelings in the last year was little altered.

In the original sub-model, there was evidence that the effect of prompted stress events in the last year varied with age – an increasing number of events was associated with non-linear decreased risk amongst younger individuals but increased risk amongst older individuals (Section 6.5.4, above). The direction of these effects persisted after controlling for a number of confounders from other sub-models (**Table 6.8.3**). The adjusted stress effect estimates were more strongly significantly associated with zoster risk in the final model, but the patterns of risk remained non-linear.

The effect of current illness was little altered in the combined model (**Table 6.8.1**). When illnesses or treatments leading to altered micronutrient availability or requirement were substituted for current illness, they were associated with more than a three-fold increased risk of zoster (**Table 6.8.2**). However, serious infections in the last six months were not significantly associated with zoster in the combined model (OR=1.55, 95%CI=0.62-3.86; p=0.352).

Ethnicity and time since varicella: individuals of Afro-Caribbean ethnicity were at half the risk of zoster on univariable analysis compared to individuals of White ethnicity. The protective effect disappeared after adjusting for other variables in the combined model (**Table 6.8.1**). This was investigated further by recoding ethnicity as a binary variable (Afro-Caribbean ethnicity vs. not), and carrying out analyses to identify proximal variables in the causal pathway between ethnicity and zoster risk. The results are summarised in **Figure 6.5** (overleaf). After adjusting for child & varicella contacts, the protective effect of Afro-Caribbean ethnicity was weakened from 0.49 (95% CI: 0.23-1.03) to 0.65 (0.28-1.48) – most of the confounding was due to contacts with specific children not living in the household (extended family and friends' children). The effect was further weakened to 0.78 (0.32-1.89) after adjusting for childhood UVR exposure, and to 0.93 (0.37-2.32) after adding fresh fruit intake to the model. Addition of smoking marginally weakened the effect to 0.96 (0.38-2.47). Recent stress, current illness, and number of years since contracting varicella appeared to have no or slight negative confounding effect on ethnicity. After adding these variables, the final adjusted effect estimate for ethnicity was 0.81 (0.30-2.25).

Figure 6.5: Univariable & adjusted estimates of the effect of ethnicity on risk of zoster



Recent acquisition of varicella remained strongly protective against zoster in the combined model (**Table 6.8.1**). The small number of individuals (one case and 11 controls) who had acquired varicella within the last ten years were at one twentieth the risk of zoster compared to those who had acquired infection at least 50 years ago, even after adjusting for other variables.

Physical trauma: recent trauma to the site of subsequent rash remained significantly associated with risk of zoster in the combined model (OR=20.14, 95%CI=2.27-178.33). However, the variable was not retained in the model, because the findings were thought to be affected by recall bias, did not confound any of the other variables of interest, and numbers with recent trauma to the site of rash were very small.

Smoking: smoking was considered only as a confounder in these analyses. However, it was noted that in the combined model current smokers were at approximately one third the risk of zoster compared to non-smokers (OR=0.35, 95%CI=0.19-0.65, p=0.0008)

6.8.3 Discussion

Most variables that were significantly associated with risk of zoster in sub-models had a similar pattern of risk in the combined model. A few variables (such as fresh fruit intake and childhood non-holiday UVR exposure) had greater magnitude of effect after adjusting for negative confounding by variables from other sub-models. The presence of negative confounders in this dataset raises the question as to whether the effect of some variables that were not associated with zoster on univariable analysis were also negatively confounded. This was investigated by re-checking some of these variables in the combined model, none of which became significantly associated with risk of zoster.

Of the major variables of interest, only Afro-Caribbean ethnicity was no longer significantly associated with zoster in the final analysis. This was protective in subgroup analyses, and previous researchers have suggested that this might be due to late age at varicella due to childhood residence in a late-onset varicella country.[81] In this population, childhood country of residence did not explain the protective effect, but collection of a wide variety of potential risk factors for zoster enabled analysis of proximal protective factors. Protection appeared to be largely mediated by increased social contacts with children, high intake of fresh fruits, and UVR exposure in childhood. The latter was slightly protective because many individuals of Afro-Caribbean ethnicity were exposed to the highest quintile of UVR exposure in childhood, whereas their matched controls mostly experienced the second, third

and fourth level of exposure. As childhood UVR exposure was associated with a quadratic pattern of zoster risk, individuals with the highest exposure levels were at lower risk of zoster compared with those at intermediate levels.

Current smokers were at lower risk of zoster after adjusting for other confounders. This is consistent with the finding of Schmader *et al*, who reported that elderly smokers were at half the risk of zoster compared to non-smokers after adjusting for the effects of age, sex, ethnicity, stress and current illness.[80] It is not clear why smoking should decrease zoster risk, given that nicotine intake has been associated with depression of cell-mediated immunity.[313,314] This might be partially explained if smokers were at similar risk of zoster compared to non-smokers, but had different recall of zoster risk factors. For example, smokers might under-report their fruit intake (a protective factor), or over-report stressful events (associated with increased risk). If this were so, smoking might appear to be protective after adjusting for these factors.

The combined model contained a large number of variables. This was consistent with the aims of the study, which were to investigate the independent effects of a range of risk factors for zoster. However, when focusing on the effects of a sub-group of risk factors, other variables in the model could be restricted to those that confounded these factors. For example, when examining the effects of child and varicella contacts on risk of zoster, most confounding variables were dealt with in sub-model analyses – only one other major confounder (unprompted stress events) was identified in the combined model, and this only confounded one of the child contact variables (occupational contacts with ill children). By restricting the number of other variables in the model when testing hypotheses relating to specific risk factors for zoster, more precise effect estimates for these factors could be obtained.

These analyses have identified a number of risk factors and protective factors for zoster, some of which were restricted to specific age groups. In the next Chapter, findings are summarised, potential limitations of the study are considered, and the research and public health implications of the findings are discussed.

7.1 SUMMARY OF FINDINGS

In this Section, the main findings of the study are considered in the context of what was already known about the topic before the study, and what the study has added.

7.1.1 Contacts with children and cases of varicella

What was already known: There were a lack of data to support or refute Hope-Simpson's hypothesis that exogenous boosting of cell-mediated immunity was important in maintaining VZV latency.[34] Two studies provided limited evidence that individuals with occupational contact with ill children were at lower risk of zoster,[106,107] and one study showed that immunosuppressed children were protected against zoster if there was a case of varicella in the household.[108]

What this study adds: contacts with children appear to protect individuals without underlying immunosuppression against zoster. A hierarchical analysis demonstrated that this protection is probably mediated by increasing exposure to cases of varicella. This supports Hope-Simpson's hypothesis, extends the findings of Gershon *et al* to a general population, and quantifies the degree of protection – the most heavily exposed individuals were at approximately one fifth the risk of zoster compared to unexposed individuals. These findings have since been supported by an analysis of the fourth Morbidity Statistics in General Practice study, which showed that individuals who had a child currently living in the household had a significantly lower incidence of zoster in one year of follow-up compared to individuals who did not live with children.[350]

7.1.2 Ethnic origin and country of birth

What was already known: one study found that elderly Black Americans were at lower risk of zoster compared to White Americans.[61] It was suggested that this might result from later age at varicella, due to childhood residence in a late-onset varicella (LV) country.[81] This hypothesis had not been formally tested.

What this study adds: consistent with previous research, this study showed that individuals of Afro-Caribbean origin were at less than half the risk of zoster compared to White individuals. However, neither childhood residence in a LV country nor late acquisition of varicella

explained the protection associated with Afro-Caribbean ethnicity, although late varicella was associated with protection against zoster. From the wide variety of information collected, it was possible to ascertain the proximal determinants of protection associated with ethnicity. These included multiple child contacts (leading to multiple varicella contacts), and high fresh fruit intake.

7.1.3 Micronutrient and food intake

What was already known: micronutrients are essential for cell-mediated immune functioning, and micronutrient deficiencies lead to immunosuppression. The elderly often have multiple micronutrient deficiencies, and so micronutrient status could be a determinant of both the loss of cell-mediated immunity with age and of zoster. Randomised controlled trials of micronutrient supplementation in the elderly have shown some evidence of improvement in cell-mediated immune functioning, but few studies have examined whether this results in increased protection against infections, and none have investigated zoster specifically.

What this study adds: intakes of individual micronutrients were not associated with zoster risk, but older individuals with relatively low combined micronutrient intake were at higher risk of zoster. This suggests that multiple micronutrient deficiencies may be a risk factor for immunosenescence. There was a strong, graded association between low fresh fruit intake and increasing risk of zoster in individuals of all ages. Fruit contains a mix of nutrients that may act together to maintain immune health.

7.1.4 Ultraviolet radiation (UVR) exposure

What was already known: acute UVR exposure results in local and systemic immunosuppression. Acute exposure is a risk factor for herpes simplex virus reactivation, but little else is known about risks of infection associated with either acute or long-term UVR exposure. A few studies have reported increased incidence of zoster in the warmer months, but no analytical studies of the effect of UVR exposure on risk of zoster have been reported.

What this study adds: There was a strong increased risk of zoster associated with childhood non-holiday UVR exposure. Amongst younger individuals, there was some evidence that childhood holiday UVR exposure also increased the risk of zoster. These childhood exposures were associated with a quadratic pattern of risk of zoster, similar to the patterns seen in previous studies of basal cell carcinoma. One explanation of these findings is that early UVR exposure 'programmes' the immune system to respond less robustly to subsequent microbial

challenges. There was also some evidence that high total UVR exposure in the month before rash onset was associated with increased risk of zoster, but the risks associated with increasing exposure showed no clear pattern.

7.1.5 Stress and illness

What was already known: psychological stress can lead to depression of cell-mediated immunity, and may increase susceptibility to infections. A case-control and a cohort study investigating the effect of stress on zoster reported that 1) individuals with zoster experienced significantly more major life events in the six months before rash compared to individuals without zoster, and significantly more negatively-perceived major events in the two months before rash,[153] and 2) there was some evidence that individuals who experienced negatively-perceived life events were at increased risk of subsequent zoster.[80] Neither study had sufficient power to investigate thoroughly the risks associated with stress. In the cohort study, individuals who self-rated their health as 'excellent' were at one half the risk of zoster compared with other individuals.[80] Individuals with diminished cell-mediated immunity due to immunosuppressive conditions or therapies are at increased risk of zoster. However, the effect of other illnesses on risk of zoster is unknown.

What this study adds: individuals who experienced incident major stress events in the two months before rash onset were at more than twice the risk of zoster compared to those who did not experience an event. Amongst older individuals, stressful events in the last 12 months were also associated with increased zoster risk. Other stressful events volunteered by participants in response to open questioning were associated with increased risk for both periods. These findings are consistent with previous studies, provide information on the importance of incident (as opposed to prevalent) events, and demonstrate that recent events may increase risk of zoster amongst younger as well as older individuals. Current medical conditions were associated with a 1.7-fold increased risk of zoster, and illnesses associated with altered micronutrient requirement or availability with a 3.6-fold increased risk, independent of reported stress.

7.1.6 Physical trauma

What was already known: previous case reports and case series suggested that mechanical trauma may result in reactivation of latent VZV. None of these studies employed controls to assess the frequency of trauma in the population from which the cases arose.

What this study adds: individuals who received trauma in the month before rash onset to the same site as subsequent rash were at eighteen times the risk of zoster compared to those without a history of trauma to the site, although the risk estimate had a wide 95% confidence interval. This suggests that trauma may increase the risk of zoster via direct stimulation of the peripheral nerve.

7.2 STRENGTHS OF THE STUDY

This study is the first to use robust epidemiological methods to investigate a wide range of risk factors for zoster. There were a number of advantages in using a population-based, matched case-control study design. These included:

1. *Advantages of the case-control design:* although the lifetime risk of zoster is estimated at 23-30%, annual incidences are relatively low. A cohort study would have been expensive and time-consuming, because it would have involved recruiting, interviewing and following up a large number of individuals. In contrast, a case-control design was efficient in terms of both sample size and logistics. This increased efficiency was accentuated by the decision to include younger individuals, who are a group at lower risk of zoster. Inclusion of younger individuals allowed investigation of potential determinants of immunosenescence - risk factors for zoster that were found amongst older but not younger individuals. In addition, the study included examination of a wide range of exposures, for which a case-control study is particularly well-suited.

2. *Advantages of a population-based study:* only a small proportion of zoster cases are seen in hospital settings. A hospital-based study would have enrolled cases that may have been atypical with respect to the exposures of interest, and would have raised difficulties in selecting an appropriate control group. Individuals with zoster are highly likely to consult their GP, and so use of a general-practice based study a) allowed recruitment of a more representative group of cases and b) identified a source of controls that represented the population from which the cases arose.

3. *Advantages of matching:* matching further increased the efficiency of the study by dealing at the design stage with the major confounding effect of age. Individual matching resulted in selection of controls who were very similar to cases with respect to age (mean difference: 4.7 days). Matching for sex removed potential confounding by sex, and facilitated some analyses – for example, comparing intake of foods within matched age-sex triplets decreased unmeasured variation between cases and controls in the portion sizes consumed of specific foods. Matching by practice meant that a) cases and matched

controls were likely to have equal access to their GP, and b) any controls who developed zoster were as likely to be reported by GPs as previous cases had been.

There were strengths associated with other aspects of the study methodology. Firstly, response rates were good for both cases (94%) and controls (at least 82.5%). High participation was attributed to a number of issues:

a) The study was endorsed by the GPs, who made initial contact with cases in person. The initial letter sent to most controls was also signed by their GP;

b) Attempts were made to actively involve all practice staff in the study;

c) The PI followed up the initial invitation letter with a personal telephone call or visit before enrolling individuals, so that they could discuss concerns about participation;

d) The PI made at least four follow-up attempts to contact individuals, by telephone and/or in person at different times of day over a period of time. Additional strategies (such as seeking information from neighbours) were used to ascertain participants' whereabouts;

e) The PI visited individuals mostly in their own homes, so as to minimise any inconvenience to them;

f) It was stressed in all communications that participation comprised a single interview, with no need for follow-up.

Secondly, there were advantages to the principal investigator (PI) undertaking all aspects of the fieldwork. This reduced the costs of the study considerably, eliminated inter-person variability in interviews, enabled the PI to assess participants' beliefs with respect to zoster that may have led to recall bias, and resulted in a very detailed understanding of the strengths and limitations of the questionnaire.

7.3 POTENTIAL LIMITATIONS OF THE STUDY

The study methodology enabled identification of many risk factors for zoster. However, aspects of the methodology might have influenced the study findings in other ways. Potential limitations of the study have already been discussed in the descriptive results (Chapter 5) and for specific analyses (Chapter 6). What follows is a summary of these discussions, considering the study as a whole.

7.3.1 Selection bias

Potential sources of selection bias included:

1. *Exclusion of cases who did not consult their GP*: Zoster results in significant acute morbidity, and it is highly likely that cases consult their GPs about their symptoms. However, zoster occasionally presents with minimal rash and pain. Some very mild cases were seen in this study, but others may not have presented to their GP. As controls were not restricted to individuals who were known to seek treatment for minor symptoms, it is possible that cases and controls came from slightly different populations, but this is unlikely to have introduced significant bias. A second consideration is that severe cases may have presented initially elsewhere, such as at hospital. These cases were likely to have their care transferred to their general practice. In this study, two severe cases who initially consulted an out-of-hours deputising service and an Accident & Emergency Department were identified for inclusion when they made follow-up visits to their GP. Hand-searching of paper records of deputising service billings in two practices did not reveal any additional cases.

2. *Underreporting of cases by GPs:* not all cases were reported by practices, despite a variety of ongoing reminders. General underreporting of cases increased the duration of fieldwork but was unlikely to have led to bias, because controls were matched to cases by practice. However, bias could have arisen if GPs were more likely to report cases that fitted the study hypotheses. As outlined in Chapter 5, this was considered unlikely, because a) the study objectives were only briefly discussed with GPs, and it was stressed that a wide variety of risk factors were being examined; b) some practices as a whole reported fewer cases over time (see **Table 5.1**); c) analysis of cases that were reported only after a second consultation and searches of partially computerised records in some practices revealed that underreporting was due to specific GPs failing to report any case, rather than selective reporting of cases. The drop in reporting over time by some practices could have introduced some bias if controls who developed zoster towards the end of the study were less likely to be reported compared to a case from the same practice who presented at the beginning of the study. However, controls who participated in the study were told that it was continuing for approximately 18 months, and they were mostly very interested in the study findings. It is therefore likely that any control who developed zoster would have either contacted the PI or mentioned the study to their GP, prompting the practice to report them as a case. One individual was interviewed as a control, and subsequently was reported and included as a case.

3. *Refusal by cases and controls to participate:* participation rates were high, and the

eligibility of individuals who refused to take part was not ascertained. Nevertheless, some bias may have been introduced if those who refused (4% of potentially eligible cases and 12.8% of potentially eligible controls) were different from those who participated with respect to the exposures of interest. This may have applied to stress events and illness, as four cases and 22 controls cited stressful events or ill-health as their reason for not participating.

4. *Other non-participation by cases and controls:* four (1.4%) of potentially eligible cases and 28 (4.7%) of potentially eligible controls either could not be contacted after multiple attempts, failed to attend the interview on two occasions, or were otherwise unavailable. It is likely that some of these individuals had moved away from the area. However, some may have been eligible for the study, and may have differed in exposure history from participants – for example, those who were unavailable because they were on extended holidays may have had higher UVR exposures. Again, any bias introduced is likely to have been minor, due to the small numbers involved.

7.3.2 Information bias

Recall bias: this is a potential problem in all case-control studies. In this study, attempts were made to minimise recall bias by not disclosing the exact study hypotheses and by asking standardised questions in a uniform way. As the PI administered all the questionnaires, much was learned about cases' beliefs with respect to risk factors for zoster. For example, many cases believed that zoster resulted from recent contact with cases of varicella. This may have resulted in cases remembering their child and varicella contacts better than controls, thus underestimating the strength of protection associated with these contacts (Chapter 6, Section 6.1.6). Another common belief was that zoster was due to 'nerves'. This may have caused cases to over-report recent stressful events compared to controls, causing an overestimate of the risk associated with stressful events. Such over-reporting may particularly have applied to information gathered from the 'open' question about stress, categorised in the analyses as 'unprompted' events (Section 6.5.2). Effect estimates for recent trauma may also have been affected by recall bias. On the other hand, there was no evidence that cases considered UVR exposure or intake of specific foods to be risk factors for zoster, and the risk of zoster associated with low fruit intake did not appear to result from underreporting of food intake in general (Chapter 6, Section 6.3.4).

Interviewer bias: the PI administered all the interviews, and so there was no masking of either the hypothesis or the case/control status of participants. If other interviewers had been used, it

would still have been very difficult to mask the case-control status of participants. Masking interviewers to the hypothesised direction of effect would have been possible for some of the exposures, but the underlying hypothesis for variables such as stress would probably have been obvious. Attempts were made to minimise information bias for exposure data by using a structured questionnaire with standard prompting questions, and it is very unlikely that the strong dose-response effects seen for a number of variables could have resulted from information bias. Data on the clinical presentation of zoster in cases were recorded at the beginning of the questionnaire, before ascertaining information on exposures.

Other differential misclassification of exposure: as outlined in Chapter 5 (Section 5.5), 22 (4.5%) controls were interviewed more than 90 days after cases. Most questions (for example for food intake or child contacts) referred to 'usual' exposure in the last year, and this was unlikely to be systematically affected by delays in interviewing controls. However, events occurring in the month before rash onset may have been recalled differently by cases and controls, because cases were interviewed soon after they developed rash but controls were interviewed some time later. Individuals were not asked specifically about events occurring within this period – information was sought on all events (such as holidays) in the previous year, and these were subsequently categorised by the PI as falling within the one or two-month period before rash onset. The delay is unlikely to have introduced differential misclassification for major events such as deaths or serious illness in the family, which controls would have remembered. However, it may have introduced greater misclassification amongst controls of less major events or exposures that involved estimation of time spent outdoors or frequency of consumption. For example, a control interviewed 90 days after a case may have forgotten a varicella contact that occurred 3-4 months previously, whereas the case might remember a contact that occurred within the last month. Such differential misclassification would result in an underestimate of the protective effect of these contacts. Similarly, a control might remember less accurately the time s/he spent outdoors on a holiday four months ago compared to a case who went on holiday in the last month. The direction of bias introduced by this misclassification would depend on whether the delay caused controls to overestimate or underestimate their exposure.

Classification of unconfirmed cases into 'probable' or 'possible' was carried out at the end of the study, without referring to individuals' exposure histories. Therefore, this is unlikely to have introduced major bias.

7.3.3 Non-differential misclassification

Misclassification of exposures – some misclassification of exposures such as micronutrient intake or UVR exposure was inevitable. For example, accurate measurement of micronutrient intake was limited by misclassification of frequency of food consumption, choice of a single food item to represent an entire food group, imperfect nutrient database information, and possible differences between cases and matched controls in portion sizes consumed (Chapter 6, Section 6.3.6). Both the FFQ and UVR data allowed ranking of individuals within populations, rather than accurate estimates of absolute intake or exposure. Respondents were categorised into quintiles of exposure, and this may have reduced misclassification. Nevertheless, non-differential misclassification of exposure may have resulted in some cases and controls being categorised into the wrong quintile of exposure, resulting in under-estimation of effects.

Misclassification of cases: a specific case definition of zoster was used, in order to exclude cases of HSV infection. As a result, a few of the 'possible' cases may have been very mild cases of zoster. Exclusion of these cases from analyses was unlikely to introduce bias, because they were not included as controls. However, this may have limited the generalisability of the study to cases of zoster with a given threshold of clinical severity. Despite the stringency of the zoster case definition, a few 'probable' zoster cases could have had atypical HSV infection. Inclusion of cases of HSV infection would result in an underestimate of the effect of exposures on risk of zoster, providing that these exposures were not associated with risk of HSV infection. However, if the exposures were risk factors for HSV infection, there might be overestimation of the estimated association with zoster. For example, acute UVR exposure is known to increase the risk of immediate HSV reactivation.[234-237] Therefore, if cases of HSV were mistakenly included as zoster, this might explain some of the apparent association seen between UVR exposure in the month before rash onset and increased risk of zoster. Any bias introduced in this way is likely to have been minor, as there were probably few cases of HSV infection included in analyses. Potential bias was investigated further by restricting analyses of recent UVR exposure and of child contacts to 'confirmed' cases and their matched controls. Results of these analyses suggested that the effects found were not attributable to undiagnosed HSV infection (**Table 6.4.14** and **Table 6.1.5**).

Misclassification of controls: controls were individuals with no self-reported history of zoster. If controls with past zoster were included, this might decrease the strength of association for historical exposures (such as UVR exposure in childhood), because controls

with past zoster may have developed zoster as a result of these exposures. Inclusion of these controls might also lead to an underestimate of the association with recent exposures (for example, usual diet), if their level of exposure had not changed in the years since they developed zoster. However, it is unlikely that controls had a history of zoster, as use of self-reporting for zoster has been shown to be highly sensitive.[283]

7.3.4 Residual confounding

Individual matching dealt with major confounding effect of age, and potential confounding by sex. The extensive data collection for this study ensured that it was possible to control for a wide variety of potential confounders. Nevertheless, misclassification of exposures that acted as confounders may have resulted in a degree of residual confounding. In addition, other unidentified confounders may have existed for some exposures. For example, the apparent protective effect of fruit intake may have been partly due to the confounding effect of physical exercise.[356]

Another potential confounder that needs consideration in this study is undiagnosed or undisclosed HIV infection. This might confound some associations because a) it was more likely to occur amongst cases compared to controls due to the increased risk of zoster amongst HIV-infected individuals, and b) it might be associated with some exposures – for example, homosexual men (who might be more likely to be HIV-positive) could have fewer child contacts. Practices were asked not to report cases with HIV infection by name, so that they would not be approached by the PI to take part in the study. Also, individuals of African ethnicity (a group at high risk of HIV infection in this population) were excluded from analyses. Therefore, it is likely that the proportion of individuals with undiagnosed HIV infection in this study was small. The effect of undiagnosed HIV infection on child contact estimates was also examined by restricting datasets to two subgroups at low risk of HIV infection in this population - women and older individuals. The results of these analyses suggest that HIV infection was not a major confounder of the protective effects of child contacts.

7.3.5 Multiple comparisons

The analysis of this study involved multiple comparisons. The concern with this is that some 'statistically significant' associations could have occurred by chance. Although some researchers use stringent p values to represent 'significance' under these circumstances or inflate p values according to the number of comparisons made, this can result in a reduction of

type I errors at the expense of an increase in type II errors, and it has been argued that chance might not be the likeliest explanation for any of the associations observed.[369] In this study, analyses were carried out to test stated hypotheses with plausible underlying biological mechanisms, and no corrections were undertaken for multiple comparisons. Chance is unlikely to explain the associations for those exposures where strong dose-response effects were seen or where highly statistically significant findings were obtained. Throughout the analyses, the number of comparisons that were made are clearly presented.

7.3.6 Reverse causality

A general concern in case-control studies is that individuals who develop disease subsequently change their exposure. However, reverse causality is unlikely to explain the findings in this study. Firstly, the majority of cases were interviewed within two weeks of rash onset. Secondly, participants were told throughout the interview that the exposures before rash onset were being sought. For example, the number of child contacts was calculated using the average frequency before onset of rash, not the frequency in the last few days (Chapter 6, Section 6.1.6).

7.4 IMPLICATIONS FOR FUTURE RESEARCH

This study identified new determinants for zoster. The findings will need to be replicated in other studies, which can focus on specific exposures in more detail. Future studies will also be able to take into account a range of newly defined potential confounders or proximal determinants of zoster risk. For example, studies of the association between ethnicity and zoster will need to consider not only country of birth and age at varicella as factors on the causal pathway between ethnicity and zoster, but also exposures such as diet and child contacts.

It is important to estimate the impact of varicella vaccination on incidence of zoster. This requires information on both the magnitude of protection afforded by a specified level of child or varicella contacts, and the prevalence of these exposures in the population of interest. If varicella contacts are to be used in impact calculations, further studies can be set up to determine the prevalence of exposure to varicella in the specified population. However, child contact data might provide more useful information, because they represent both known and unknown varicella contacts. Frequency of social child contacts will also vary in different populations, due to differences in social mixing patterns with children. Therefore, the child contact odds ratios derived from this study could be combined with estimates of the number of

individuals in the population of interest in each of the exposure strata used in this study. Alternatively, population-specific odds ratios could be re-estimated, using quintiles of exposure to children in that population. The PI has been recently approached by the National Centre for Immunisation Research in Australia, asking for assistance in planning a similar study on child and varicella contacts on the risk of zoster.

Once effect- and prevalence estimates for child and varicella contacts have been obtained, these can be used in mathematical models to predict increases in zoster incidence resulting from loss of exogenous exposures after introduction of childhood varicella vaccination. These predictions can then inform evaluations of the cost-effectiveness of introducing varicella vaccination. In countries where vaccination has already been introduced, direct measurement of the potential impact of widespread childhood varicella vaccination on incidence of zoster has been hindered by limited surveillance of zoster. As a result of the recent debate about exogenous boosting, the US Centers for Disease Control and Prevention (CDC) have now set up sentinel sites in two areas, to monitor incidence of both varicella and zoster.[370] Uptake of vaccination was initially slow, but any effect on zoster incidence should become apparent once levels of circulating wild-type virus have fallen sufficiently.

Further epidemiological data are needed on the effects of UVR exposure on susceptibility to infections. The wealth of data collected in this study will be analysed at a future date to see whether long-term UVR exposure affects the risk of zoster, and whether it modifies the effects of childhood and recent exposure. Measurement of past UVR exposure needs better instruments, so that misclassification of exposure is minimised and administration and coding of questionnaires is less labour intensive. Future studies of the effects of childhood UVR exposure on risk of zoster will also need to collect data on potential confounding variables, such as childhood vaccination and nutritional status. A cohort study of childhood exposure might allow a more accurate assessment of UVR dosage (for example, using personal dosimeters), and could collect detailed information on potential confounders. However, this would necessitate a large study and very long duration of follow-up. It would be feasible to set up cross-sectional studies of adolescents to examine whether childhood UVR exposure programmes the immune system. This could involve use of questionnaires to determine past UVR exposure, history of infections and data on confounding variables, together with measurement of immune parameters.

Future studies of the effect of diet on risk of zoster might collect more data on fruit and vegetable intake, including information on portion size and details of consumption of vegetables in different forms (for example, asking separately about cooked and raw carrot

consumption). This might clarify whether variability in these factors explained why there was only a relatively weak association between vegetable intake and risk of zoster in this study, but a strong association with fruit intake. Again, if there was scope to explore the effect of diet in existing large cohort studies, methodologies such as seven-day food diaries could be used. These methods might give a more useful assessment of actual (as opposed to relative) intake.[304] The finding that elderly individuals with relatively low combined micronutrient intake were at increased risk of zoster suggests that these individuals might benefit from supplementation. Future randomised controlled trials could assess the effect of micronutrient supplementation separately in participants who are deficient in micronutrients, so see whether the effects differ according to baseline nutritional status.

The immunological changes that characterise declining cell-mediated immunity in the elderly have been well described.[132] However, many studies investigating the ageing immune system have been cross-sectional, and relatively little is known about either the average age at onset of immunosenescence or its determinants. The present research split the study population into 'younger' (<60 years) and 'older' (≥60 years) individuals, and identified two possible determinants of immunosenescence – low combined micronutrient intake and a high number of stressful events in the last 12 months. Future studies may wish to use more finely graded age groupings, and look for trends in risk with increasing age.

Finally, it is widely assumed that zoster can be diagnosed on clinical grounds. Although atypical HSV and zoster presentations are probably rare in immunocompetent individuals, there are a lack of good quality data with which to assess their frequency. Use of polymerase chain reaction in studies of individuals with zosteriform rashes and typical 'HSV' vesicular rashes will allow an accurate assessment of the sensitivity and specificity of clinical case definitions for zoster.

7.5 IMPLICATIONS FOR PRACTICE

The findings on the effect of dietary intake on risk of zoster are of public health importance. Although the underlying mechanism by which high fruit intake may protect against zoster was not elucidated, the protective effects associated with fruit consumption provides further support for the recommendation from the World Health Organisation and from national governments to eat five portions of fruit and/or vegetables per day.[371-373] As outlined in Chapter 2, multiple micronutrient deficiencies are common amongst older individuals, particularly those who are institutionalised. There are insufficient data from randomised controlled trials to show that

micronutrient supplementation lowers susceptibility to infection, and so it is premature to suggest that supplements be provided routinely to the elderly to protect against zoster. Nevertheless, elderly individuals with relatively low micronutrient intake were at increased risk of zoster in this study, and selective supplementation of high-risk individuals may be considered as a cost-effective way to decrease morbidity.

National and international organisations have expressed concerns that depletion of the ozone layer and the resulting increased exposure to UVR could result in a range of adverse health effects, including altered susceptibility to infectious diseases.[214,374-376] Numerous national public health campaigns have been set up to advise individuals to protect themselves from the sun.[377-380] These campaigns include specific programmes targeted at reducing UVR exposure in children, but they have all focussed on the need to prevent skin cancer. The findings from the present study indicate suggest that campaigns that encourage parents to protect their children against excessive UVR exposure may also help to reduce future zoster incidence.

The study findings on the protective effect of exogenous VZV exposures against zoster are timely, because they were reported at a time when many European and non-European countries were considering the introduction of varicella vaccination. The findings were forwarded to the Department of Health's Joint Committee on Vaccines and Immunisation, where they informed the Committee's decision on whether to add varicella vaccine to the childhood schedule (A. Hall, personal communication). Since the study was published, a number of other European countries have decided against introducing routine varicella vaccination (data presented at the 7th meeting of the VZV Research Foundation European Working Group on Varicella, November 2002). In countries such as the USA where childhood vaccination has already been introduced, there is now heightened interest in the results of the current multi-centre trial set up to ascertain whether varicella vaccination protects the elderly against zoster.[381] If the results of the trial indicate that vaccination is effective, both existing and planned childhood varicella vaccination programmes will need to consider whether to add vaccination of older subjects to the vaccination schedule.

In conclusion, this thesis has reported the design and results of the first population-based study set up to investigate multiple risk factors for zoster amongst individuals without underlying immunosuppression. The results confirmed previous findings on the effect of stress on risk of zoster, elucidated the mechanisms by which some ethnic groups may be at lower risk of zoster, and identified new determinants of zoster, including exogenous contacts with varicella cases, UVR exposure in childhood, and fruit intake. The inclusion of both older and younger individuals allowed investigation of potential determinants of immunosenescence, and

identified two such risk factors – combined micronutrient intake and stressful events. The study findings have had a significant impact on international varicella vaccination policy, and are likely to inform both future research and public health practice.

9. Tables for analyses reported in Chapter 6

(Table 6.6.1 – Table 6.8.3)

Table 6.1.1: Univariable analyses of the effect of contacts with cases of varicella or zoster in the last 10 years on the risk of zoster (n=729)

Contacts in last 10y	CASES n (%)	CONTROLS n (%)	Univariable OR (95% CI)	
*No. of **varicella** contacts:*				
None	179 (73.4)	283 (58.4)	1.00	
1	34 (13.9)	74 (15.3)	0.67 (0.41-1.08)	$p < 0.0001$
2	20 (8.2)	45 (9.3)	0.61 (0.34-1.09)	*p (trend)* < 0.0001
3-4	6 (2.5)	44 (9.1)	0.15 (0.06-0.39)	
5+	5 (2.0)	39 (8.0)	0.14 (0.05-0.39)	
*No. of **zoster** contacts:*				
None	189 (77.5)	338 (69.7)	1.00	$p = 0.052$
1	44 (18.0)	110 (22.7)	0.71 (0.48-1.05)	*p (trend)* = 0.015
2+	11 (4.5)	37 (7.6)	0.51 (0.25-1.04)	

Table 6.1.2: Univariable analyses of the effect of contacts with children in the last 10 years on the risk of zoster (n=729)

Contacts in last 10y	CASES n (%)	CONTROLS n (%)	Univariable OR (95% CI)	
Contacts with a few children				
Total no. of child-day contacts with children living in the household:				
None	202 (82·8)	355 (73·2)	1·00	
7-2550[1]	27 (11·1)	65 (13·4)	0·62 (0·37-1·05)	
2551-14901	15 (6·1)	65 (13·4)	0·34 (0·18-0·64)	p=0·001 $p(t)$=0·0002*
Childcare work:				
None	233 (95·5)	436 (89·9)	1·00	
≤ 5 years duration	10 (4·1)	28 (5·8)	0·37 (0·13-1·06)	
> 5 years duration	1 (0·4)	21 (4·3)	0·06 (0·01-0·50)	p=0·001 $p(t)$=0·0001*
Contacts with multiple children				
Total no. of social contacts with specific children not living in the household:				
None	30 (12·3)	49 (10·1)	1·00	
2-107[1]	60 (24·6)	87 (17·9)	1·02 (0·59-1·81)	
108-420	53 (21·7)	88 (18·1)	0·91 (0·51-1·62)	
421-1334	52 (21·3)	87 (18·9)	0·89 (0·49-1·63)	
1335-3457	30 (12·3)	87 (18·9)	0·53 (0·28-0·98)	
3458-32631	19 (7·8)	87 (17·9)	0·30 (0·14-0·63)	p=0·0003 $p(t)$ <0.0001*
Total no. of social contacts with children in groups:				
None	197 (80·7)	308 (63·5)	1·00	
6-550[1]	24 (9·8)	59 (12·2)	0·63 (0·38-1·06)	
551-3652	16 (6·6)	59 (12·1)	0·32 (0·17-0·62)	
3653-45023	7 (2·9)	59 (12·2)	0·12 (0·06-0·35)	p<0·0001 $p(t)$ <0.0001*
Occupational contact with multiple ill children:				
None	241 (98·8)	460 (94·8)	1·00	
≤ 5 years duration	2 (0·8)	14 (2·9)	0·26 (0·06-1·17)	
> 5 years duration	1 (0·4)	11 (2·3)	0·17 (0·02-1·29)	p=0·015 $p(t)$=0.004*
Occupational contact with multiple well children:				
None	209 (85.3)	419 (86.4)	1.00	
≤ 5 years duration	20 (8.6)	36 (7.4)	1.11 (0.62-1.99)	
> 5 years duration	15 (6.1)	30 (6.2)	1.01 (0.52-1.96)	p = 0.941 $p(t)$ = 0.859*

[1] Quantiles of exposure – see Section 6.1.2. * *p(t)= p value for trend*

Table 6.1.3: Multivariable analysis - effects of contacts in the last ten year with a few children, before and after adjusting for multiple child contacts (n=729)

Distal variables[1]	Univariable OR (95% CI)	Adjusted for intermediate[1] social child contacts
Childcare work with a few specific children:		
None	1·00	1·00
≤ 5 years duration	0·37 (0·13-1·06)	0·94 (0·27-2·99)
> 5 years duration	0·06 (0·01-0·50)	0·19 (0·02-1·79)
	p=0·0004	p=0·214 p(t)=0·169*
Total no. of child-day contacts with children living in the household:		
None	1·00	1·00
7-2550[2]	0·62 (0·37-1·05)	0·96 (0·54-1·69)
2551-14901	0·34 (0·18-0·64)	0·71 (0·34-1·47)
	p=0·001	p=0·638 p (t)=0·403*

[1] See **Figure 6.1** and text [2] Quantiles of exposure – see Section 6.1.2 * $p(t)$ = p value for trend

Table 6.1.4: Multivariable analyses of the effects of child contacts and contacts with varicella and zoster cases in the last 10 years on the risk of zoster (n=729)

Intermediate variables[1]	Univariable OR (95% CI)	Adjusted for other intermediate variables+ varicella contacts[3]
Total no. of social contacts with specific children not living in the household:		
None	1·00	1·00
2-107[2]	1·02 (0·59-1·81)	1·03 (0·57-1·85)
108-420	0·91 (0·51-1·62)	0·94 (0·52-1·73)
421-1334	0·89 (0·49-1·63)	0·90 (0·48-1·70)
1335-3457	0·53 (0·28-0·98)	0·60 (0·30-1·17)
3458-32631	0·30 (0·14-0·63)	0·43 (0·19-0·94)
	p=0·0003	p=0·079 p(t)=0·007
Total no. of social contacts with children in groups:		
None	1·00	1·00
6-550[2]	0·63 (0·38-1·06)	0·72 (0·41-1·27)
551-3652	0·32 (0·17-0·62)	0·44 (0·22-0·89)
3653-45023	0·12 (0·06-0·35)	0·19 (0·07-0·50)
	p<0·0001	p=0·001 p(t)=0·0001
Occupational contact with multiple ill children:		
None	1·00	1·00
≤ 5 years duration	0·26 (0·06-1·17)	0·25 (0·05-1·20)
> 5 years duration	0·17 (0·02-1·29)	0·27 (0·03-2·51)
	p=0·015	p=0·062 p (t)=0·025
Proximal variables[1]		
No. of known contacts with varicella cases:	**Univariable OR**	**Adjusted for intermediate variables (above)[3]**
None	1·00	1·00
1	0·67 (0·42-1·08)	0·90 (0·54-1·52)
2	0·61 (0·34-1·09)	0·83 (0·45-1·56)
3 - 4	0·15 (0·06-0·39)	0·26 (0·10-0·72)
5+	0·14 (0·05-0·39)	0·29 (0·10-0·84)
	p<0·0001	p=0·016 p (t)=0·003
No. of known contacts with zoster cases:		**Adjusted for intermediate variables + varicella contacts[3]**
None	1·00	1·00
1	0·71 (0·48-1·05)	0·79 (0·51-1·23)
2+	0·51 (0·25-1·04)	0·92 (0·42-2·03)
	p=0·052	p=0·581

[1] See Figure 1 and text [2] Quantiles of exposure [3] Also adjusted for ethnicity *p(t) = p value for trend

Table 6.1.5: Effect of child contacts in the last 10y on the risk of zoster in study population subsets (not adjusted for contact with cases of varicella)

Variable	Odds ratio (adjusted for other variables in the Table)			
	In total study population (n=729)	In individuals aged ≥60y (n=339)	In women - all ages (n=411)	PCR+ve cases & matched controls (n=275)
Contacts with specific children not living in the household:				
None	1.00	1.00	1.00	1.00
1-107	1.04 (0.58-1.86)	1.44 (0.65-3.17)	1.13 (0.48-2.65)	1.27 (0.48-3.35)
108-420	0.89 (0.49-1.61)	1.08 (0.49-2.40)	0.82 (0.34-1.99)	1.18 (0.44-3.17)
421-1334	0.87 (0.46-1.63)	1.41 (0.61-3.25)	0.96 (0.41-2.26)	0.83 (0.29-2.35)
1335-3457	0.52 (0.27-0.99)	0.75 (0.30-1.84)	0.76 (0.30-1.93)	0.24 (0.07-0.84)
3458-32631	0.36 (0.17-0.77)	0.42 (0.12-1.39)	0.39 (0.12-1.09)	0.55 (0.15-2.02)
Contacts with children in groups:				
None	1.00	1.00	1.00	1.00
1-550	0.67 (0.39-1.15)	1.07 (0.43-2.63)	0.66 (0.31-1.40)	0.96 (0.42-2.17)
551-3652	0.36 (0.18-0.71)	0.59 (0.18-1.90)	0.32 (0.12-0.80)	0.30 (0.10-0.92)
3653-7492	0.14 (0.06-0.37)	0.15 (0.02-1.29)	0.22 (0.08-0.61)	0.09 (0.01-0.81)
Occupational contact with ill children:		**As binary variable[1]**		**As binary variable for lifetime contacts[2]**
None	1.00	1.00	1.00	1.00
≤5 years	0.22 (0.05-1.05)	0.31 (0.03-2.90)	0.17 (0.02-1.46)	0.10 (0.01-0.84)
>5 years	0.14 (0.02-1.27)		0.21 (0.02-2.16)	

[1] No cases had exposure of more than 5 years [2] No cases had exposure in the last 20 years

Table 6.1.6. Effect[1] of social contacts in the last year with specific children not living in the household on the risk of zoster, by age of cases and controls.

Contacts in last year with specific children not living in the household:	Age < 60y (n=390)	Age ≥ 60y (n=339)
None	1.00	1.00
1-11	0.32 (0.14-0.72)	1.06 (0.48-2.33)
12-52	0.55 (0.26-1.17)	2.12 (1.04-4.36)
53-155	0.47 (0.21-1.08)	1.13 (0.54-2.38)
156-381	0.28 (0.12-0.66)	0.56 (0.20-1.60)
382-3650	0.12 (0.04-0.37)	1.14 (0.44-2.96)
	p=0.0007 $p(trend)$=0.0003	p=0.139

[1] Adjusted for contacts with groups of children, occupational exposure to ill children in last 10 years, childcare 1-10y ago, ethnicity

Table 6.1.7. Effect[1] of number of contacts per child with specific children not living in the house on the risk of zoster among individuals with one or more contact in the last year, by number of specific children contacted (n=470)

	Number of specific children contacted in the last year	
Contacts per child in last year with specific children not living in the household:	1 - 3 Children (n=242)	>3 Children (n=228)
1 – 6	1.00	1.00
7 - 26	1.14 (0.54-2.41)	0.66 (0.27-1.61)
27 - 78	1.40 (0.70-2.78)	0.36 (0.14-0.88)
79 - 365	0.85 (0.39-1.91)	0.27 (0.10-0.77)
	$p(trend)$=0.925	$p(trend)$=0.0004

[1]Adjusted for number of contacts with groups of children in the last year, occupational contact with ill children in the last year, duration of childcare 1-10 years ago, ethnicity

Table 6.2.1: Univariable analyses of the effect of ethnic origin, childhood residence and varicella history on risk of zoster (n=729)

Variable	CASES n (%)	CONTROLS n (%)	OR (95% CI)		
Ethnic origin:					
White	220 (90.2)	424 (87.4)	1.00		
Afro-Caribbean	10 (4.1)	40 (8.2)	0.45 (0.20-0.96)		
Asian	7 (2.9)	9 (1.9)	1.46 (0.52-4.11)		
Other	7 (2.9)	12 (2.5)	1.09 (0.42-2.86)		
				$p = 0.130$	
Tropical childhood residence: (lat $\leq 25^0$):					
No	227 (93.0)	441 (90.9)	1.00		
Yes – mostly before 1^0 school	4 (1.6)	7 (1.4)	1.03 (0.30-3.56)		
Yes – for most of 1^0 school	13 (5.3)	37 (7.6)	0.66 (0.34-1.31)		
				$p = 0.480$	
LV childhood residence[1]*:*					
No	231 (94.7)	451 (93.0)	1.00		
Yes - mostly before 1^0 school	2 (0.8)	5 (1.0)	0.72 (0.14-3.74)		
Yes - for most/all of 1^0 school	11 (4.5)	29 (6.0)	0.72 (0.33-1.54)		
				$p = 0.628$	
Age at varicella:					
No history of varicella	96 (39.3)	157 (32.4)	1.53 (1.02-2.30)		
<1yr	2 (0.8)	2 (0.4)	2.35 (0.33-17.00)		
1 - 10yr	80 (32.8)	183 (37.7)	1.00		
11 - 20yr	9 (3.7)	26 (5.4)	0.81 (0.36-1.84)		
21 - 68yr	4 (1.6)	21 (4.3)	0.43 (0.14-1.32)		
Unknown (+ve history varicella)	53 (21.7)	96 (19.8)	1.28 (0.83-1.97)		
				$p = 0.072$	
No. of years since varicella[2]*:*					**Amongst those with a varicella history**[3]
>50 years	55 (22.5)	130 (26.8)	1.00		1.00
31 - 50 years	55 (22.5)	107 (22.1)	1.24 (0.63-2.45)		0.74 (0.21-2.63)
11 - 30 years	37 (15.2)	79 (16.3)	0.70 (0.30-1.63)		0.12 (0.01-1.27)
≤ 10 years	1 (0.4)	12 (2.5)	0.17 (0.01-1.07)		0.02 (0.00-0.56)
No history of varicella	96 (39.3)	157 (32.4)	1.42 (0.92-2.21)		-
			$p = 0.026$		$p = 0.037$
					$p(t)$=0.013

[1] Late-onset-varicella residence: Caribbean region, South India, Sri Lanka, Singapore, Malaysia, Philippines
[2] Those with a history of varicella at unknown age were re-categorised as acquiring varicella between 1-10yrs
[3] n=353 $p(t)$=p value for trend

Table 6.2.2: Multivariable analysis - effect of ethnicity and number of years since varicella on risk of zoster (n=729)

Variable	Univariable OR	Adjusted for other variable in the Table
Ethnicity:		
White	1.00	1.00
Afro-Caribbean	0.45 (0.20-0.96)	0.44 (0.21-0.92)
Asian	1.46 (0.52-4.11)	2.03 (0.66-6.23)
Other	1.09 (0.42-2.86)	1.05 (0.38-2.91)
	$p = 0.130$	$p = 0.057$
No. of years since varicella:*		
>50 years	1.00	1.00
31 - 50 years	1.24 (0.63-2.45)	1.27 (0.63-2.53)
11 - 30 years	0.70 (0.30-1.63)	0.69 (0.29-1.63)
≤ 10 years	0.12 (0.01-1.07)	0.10 (0.01-0.98)
No history of varicella	1.42 (0.92-2.21)	1.51 (0.97-2.35)
	$p = 0.026$	$p = 0.012$

* Those with a history of varicella at unknown age were re-categorised as acquiring varicella between 1-10yrs

Table 6.2.3: Multivariable analysis - effect of ethnicity and number of years since varicella on risk of zoster, excluding those with no history of varicella (n=353)

Variable	Univariable OR	Adjusted for other variable in the Table
Ethnicity:		
White	1.00	1.00
Afro-Caribbean	0.30 (0.10-0.93)	0.32 (0.10-1.00)
Asian	0.86 (0.20-3.76)	1.12 (0.23-5.35)
Other	0.54 (0.14-2.07)	0.52 (0.13-2.08)
	$p = 0.122$	$p = 0.170$
No. of years since varicella:*		
>50 years	1.00	1.00
31 - 50 years	0.74 (0.21-2.63)	0.81 (0.22-2.95)
11 - 30 years	0.12 (0.01-1.27)	0.14 (0.01-1.58)
≤ 10 years	0.02 (0.00-0.56)	0.03 (0.00-0.67)
No history of varicella	(excluded)	-
	$p = 0.037$	$p = 0.053$ p (trend) = 0.021

* Those with a history of varicella at unknown age were re-categorised as acquiring varicella between 1-10yrs

Table 6.3.1: Univariable analyses of the effect of daily micronutrient intake from food in the previous year on the risk of zoster (n=726)

Nutrient-adjusted[1] micronutrient intake	CASES n(%)	CONTROLS n(%)	OR (95% CI)	
Iron (mg):				
13.1 - 23.8	50 (20.6)	96 (19.9)	1.00	
11.7 - 13.0	49 (20.2)	97 (20.1)	0.98 (0.60-1.59)	$p = 0.934$
10.7 - 11.6	42 (17.3)	97 (20.1)	0.84 (0.51-1.39)	$p(t) = 0.865$
9.5 - 10.6	51 (21.0)	96 (19.9)	1.04 (0.64-1.70)	
5.8 - 9.4	51 (21.0)	97 (20.1)	1.02 (0.62-1.67)	
Zinc (mg):				
10.7 - 15.9	46 (18.9)	97 (20.1)	1.00	
9.8 - 10.6	50 (20.6)	97 (20.1)	1.08 (0.66-1.77)	$p = 0.456$
9.0 - 9.7	38 (15.6)	96 (19.9)	0.83 (0.49-1.39)	$p(t) = 0.577$
7.9 - 8.9	62 (25.5)	97 (20.1)	1.31 (0.82-2.08)	
4.8 - 7.8	47 (19.3)	96 (19.9)	1.04 (0.62-1.73)	
Retinol-equivalents (μg):				
2211.9 - 10420.8	47 (19.3)	97 (20.1)	1.00	
1149.0 - 2211.8	53 (21.8)	97 (20.1)	1.11 (0.69-1.77)	$p = 0.898$
931.4 - 1148.9	50 (20.6)	96 (19.9)	1.08 (0.66-1.75)	$p(t) = 0.863$
740.0 - 931.3	42 (17.3)	97 (20.1)	0.88 (0.52-1.47)	
161.0 - 739.9	51 (21.0)	96 (19.9)	1.06 (0.64-1.74)	
Vitamin B_6 (mg):				
2.6 – 4.2	45 (18.5)	96 (20.0)	1.00	
2.3 – 2.5	49 (20.2)	97 (20.0)	1.07 (0.65-1.76)	$p = 0.737$
2.1 – 2.2	43 (17.7)	97 (20.0)	0.95 (0.57-1.58)	$p(t) = 0.585$
1.8 – 2.0	59 (24.3)	97 (20.0)	1.31 (0.81-2.13)	
0.8 – 1.7	47 (19.3)	96 (20.0)	1.05 (0.62-1.76)	
Folic acid (μg):				
365.7 – 615.2	40 (16.5)	97 (20.1)	1.00	
324.4 – 365.6	48 (19.7)	97 (20.1)	1.22 (0.74-2.02)	$p = 0.763$
290.6 – 324.3	49 (20.2)	95 (19.6)	1.26 (0.76-7.09)	$p(t) = 0.267$
250.1 – 290.5	55 (22.6)	97 (20.1)	1.39 (0.85-2.29)	
111.3 – 250.0	51 (21.0)	97 (20.1)	1.29 (0.77-2.15)	
Vitamin C (mg):				
175.7 - 384.9	33 (13.6)	97 (20.1)	1.00	
130.1- 177.4	48 (19.7)	97 (20.1)	1.49 (0.88-2.53)	$p = 0.160$
97.3 - 130.0	50 (20.6)	97 (20.1)	1.56 (0.92-2.65)	$p(t) = 0.021$
68.5 - 97.2	50 (20.6)	95 (19.6)	1.60 (0.95-2.70)	
5.2 - 68.4	62 (25.5)	97 (20.1)	1.95 (1.15-3.30)	
Vitamin E (mg):				
14.0 – 30.4	46 (18.9)	97 (20.1)	1.00	
9.2 – 13.9	56 (23.1)	97 (20.1)	1.23 (0.75-2.02)	$p = 0.775$
7.1 – 9.1	52 (21.4)	97 (20.1)	1.13 (0.69-1.86)	$p(t) = 0.502$
5.4 – 7.0	47 (19.3)	96 (19.9)	0.99 (0.60-1.65)	
1.7 – 5.3	42 (17.3)	96 (19.9)	0.89 (0.53-1.51)	

[1] Derived from the residuals from the regression model of micronutrient intake and total energy intake, and the predicted micronutrient value for the mean energy intake (see Chapter 4) $p(t) = p$ value for trend

Table 6.3.2: Effect of daily micronutrient intake from food & supplements in the previous year on risk of zoster in individuals without conditions associated with micronutrient deficiency[2] (n=671)

Nutrient-adjusted[1] micronutrient intake	CASES n(%)	CONTROLS n(%)	OR (95% CI)	
Iron (mg):				
14.1 - 241.8	43 (18.9)	88 (19.8)	1.00	
12.0 - 14.0	46 (20.3)	91 (20.5)	1.05 (0.63-1.77)	$p = 0.964$
10.8 - 11.9	46 (20.3)	87 (19.6)	1.09 (0.66-1.82)	$p(t) = 0.639$
9.6 - 10.7	43 (18.9)	91 (20.5)	0.99 (0.59-1.67)	
5.6 - 9.5	49 (21.6)	87 (19.6)	1.18 (0.70-1.98)	
Zinc (mg):				
11.1 - 34.9	45 (19.8)	89 (20.1)	1.00	
10.1 - 11.0	45 (19.8)	89 (20.1)	1.00 (0.60-1.67)	$p = 0.933$
9.1 - 10.0	40 (17.6)	89 (20.1)	0.90 (0.54-1.49)	$p(t) = 0.718$
7.9 - 9.0	51 (22.5)	88 (19.8)	1.13 (0.69-1.85)	
4.8 - 7.8	46 (20.3)	89 (20.1)	1.04 (0.62-1.75)	
Retinol-equivalents (μg):				
2358.6 - 10465.0	42 (18.5)	88 (19.8)	1.00	
1519.0 - 2358.5	47 (20.7)	88 (19.8)	1.13 (0.67-1.89)	$p = 0.986$
1018.2 - 1518.9	46 (20.3)	88 (19.8)	1.09 (0.65-1.84)	$p(t) = 0.873$
777.3 - 1018.1	44 (19.4)	90 (20.3)	1.02 (0.60-1.72)	
161.7 - 777.2	48 (21.1)	90 (20.3)	1.11 (0.66-1.89)	
Vitamin B_6 (mg):				
3.2 - 309.2	40 (17.6)	87 (19.6)	1.00	
2.6 - 3.1	53 (23.4)	90 (20.3)	1.28 (0.77-2.13)	$p = 0.891$
2.2 - 2.5	47 (20.7)	90 (20.3)	1.16 (0.70-1.92)	$p(t) = 0.911$
1.9 - 2.1	45 (19.8)	93 (20.9)	1.05 (0.63-1.75)	
0.7 - 1.8	42 (18.5)	84 (18.9)	1.08 (0.62-1.87)	
Folic acid (μg):				
411.8 – 1097.3	36 (15.9)	84 (18.9)	1.00	
340.6 - 411.7	55 (24.2)	92 (20.7)	1.46 (0.86-2.47)	$p = 0.584$
299.0 - 340.5	44 (19.4)	91 (20.5)	1.17 (0.68-2.00)	$p(t) = 0.874$
254.8 - 298.9	50 (22.0)	87 (19.6)	1.40 (0.83-2.39)	
118.0 - 254.7	42 (18.5)	90 (20.3)	1.11 (0.64-1.94)	
Vitamin C (mg):				
209.4 - 1428.5	35 (15.4)	90 (20.3)	1.00	
146.1 - 209.3	53 (23.4)	87 (19.6)	1.53 (0.93-2.52)	$p = 0.196$
105.0 - 146.0	51 (22.5)	89 (20.0)	1.48 (0.86-2.52)	$p(t) = 0.462$
72.7 - 104.9	36 (15.9)	92 (20.7)	1.01 (0.59-1.77)	
12.0 - 72.6	52 (22.9)	86 (19.4)	1.57 (0.92-2.68)	
Vitamin E (mg):				
18.9 - 542.9	39 (17.2)	84 (18.9)	1.00	
11.9 - 18.8	63 (27.8)	89 (20.0)	1.54 (0.93-2.55)	$p = 0.055$
8.0 - 11.9	45 (19.8)	89 (20.0)	1.10 (0.65-1.86)	$p(t) = 0.119$
5.7 - 7.9	50 (22.0)	90 (20.3)	1.16 (0.70-1.93)	
1.9 - 5.6	30 (13.2)	92 (20.7)	0.69 (0.39-1.23)	

[1] See footnote to Table 6.3.1 [2] See text $p(t) = p$ value for trend

Table 6.3.3: Univariable analyses of the effect of combined daily micronutrient intake in the previous year on risk of zoster (n=726)

	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
a) Intakes from foods				
Total micronutrient score[1]*:*				
27 – 35	44 (18.1)	104 (21.5)	1.00	
23 – 26	44 (18.1)	97 (20.1)	1.07 (0.65-1.77)	$p = 0.655$
20 – 22	50 (20.6)	86 (17.8)	1.39 (0.84-2.31)	$p(t) = 0.289$
16 – 19	54 (22.2)	96 (19.9)	1.33 (0.82-2.15)	
7 – 15	51 (21.0)	100 (20.7)	1.23 (0.74-2.05)	
No. of micronutrients at highest level[2]*:*				
3 - 7	40 (16.5)	99 (20.5)	1.00	
2	45 (18.5)	82 (17.0)	1.36 (0.82-2.25)	$p = 0.489$
1	68 (28.0)	142 (29.4)	1.19 (0.75-1.89)	
0	90 (37.0)	160 (33.1)	1.39 (0.89-2.17)	
b) Intakes from foods & supplements:				
Total micronutrient score[1]*:*				
27 – 35	50 (20.6)	114 (23.6)	1.00	
23 – 26	47 (19.3)	84 (17.4)	1.27 (0.78-2.06)	$p = 0.906$
20 – 22	36 (14.8)	72 (14.9)	1.15 (0.68-1.92)	$p(t) = 0.605$
16 – 19	57 (23.5)	106 (22.0)	1.15 (0.73-1.83)	
7 – 15	53 (21.8)	107 (22.1)	1.19 (0.74-1.90)	
No. of micronutrients at highest level[2]*:*				
3 - 7	41 (16.9)	98 (20.3)	1.00	
2	39 (16.1)	64 (13.2)	1.46 (0.85-2.51)	$p = 0.511$
1	57 (23.5)	122 (25.3)	1.12 (0.69-1.82)	$p(t) = 0.479$
0	106 (43.5)	199 (41.2)	1.29 (0.82-2.01)	

[1]Sum of quintile scores of each micronutrient of interest (see text)
[2] At highest quintile of exposure (see text)
$p(t) = p$ value for trend

Table 6.3.4: Univariable analyses of the effect of dietary fruit and vegetable intake in the last year on risk of zoster (n=726)

Average intake in the last year	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
***Combined** fresh/frozen fruit & vegetables:*				
≥ 8 portions per day	29 (11.9)	103 (21.3)	1.00	
6 - 7 portions per day	50 (20.6)	113 (23.4)	1.76 (1.02-3.04)	$p = 0.005$
4 - 5 portions per day	86 (35.4)	151 (31.3)	2.25 (1.34-3.79)	$p(t)=0.0004$
2 - 3 portions per day	61 (25.1)	91 (18.8)	2.63 (1.51-4.60)	
≤ 1 portion per day	17 (7.0)	25 (5.2)	2.76 (1.23-6.00)	
Fresh fruit:				
> 3 portions per day	36 (14.8)	116 (24.0)	1.00	
2 - 3 portions per day	79 (32.5)	157 (32.5)	1.64 (1.03-2.60)	$p = 0.025$
5 - 7 portions per week	68 (28.0)	122 (25.3)	1.77 (1.11-2.82)	$p(t)=0.002$
1 - 4 portions per week	42 (17.3)	65 (13.5)	2.21 (1.26-3.87)	
< 1 portion per week	18 (7.4)	23 (4.8)	2.53 (1.22-5.22)	
Fresh or frozen (all colour) vegetables:				
≥ 5 portions per day	39 (16.1)	104 (21.5)	1.00	
4 portions per day	42 (17.3)	104 (21.5)	1.10 (0.65-1.87)	$p = 0.094$
3 portions per day	62 (25.5)	117 (24.2)	1.45 (0.87-2.42)	$p(t)=0.005$
1 - 2 portions per day	81 (33.3)	132 (27.3)	1.71 (1.06-2.76)	
< 1 portion per day	19 (7.8)	26 (5.4)	2.09 (1.00-4.34)	
*Fresh/ frozen **green, red or yellow** vegetables:*				
≥ 5 portions per day	4 (1.6)	27 (5.6)	1.00	
4 portions per day	15 (6.2)	39 (8.1)	2.64 (0.79-8.82)	$p = 0.055$
3 portions per day	51 (21.0)	101 (20.9)	3.51 (1.15-10.74)	$p(t)=0.011$
1 - 2 portions per day	135 (55.6)	258 (53.4)	3.66 (1.23-10.92)	
< 1 portion per day	38 (15.6)	58 (12.0)	4.39 (1.40-13.74)	

$p(t) = p$ value for trend

Table 6.3.5: Univariable analyses of the effect of daily micronutrient intake from food in the two months before rash onset on risk of zoster (n=726)

Nutrient-adjusted[1] micronutrient intake	CASES n(%)	CONTROLS n(%)	OR (95% CI)	
Iron (mg):				
13.1 - 23.9	51 (21.0)	97 (20.1)	1.00	
11.6 - 13.0	49 (20.2)	97 (20.1)	0.97 (0.60-1.58)	$p = 0.597$
10.7 - 11.5	37 (15.2)	97 (20.1)	0.73 (0.44-1.22)	$p(t) = 0.720$
9.4 - 10.6	54 (22.2)	97 (20.1)	1.08 (0.66-1.77)	
5.7 - 9.3	52 (21.4)	95 (19.6)	1.05 (0.65-1.71)	
Zinc (mg):				
10.7 – 16.2	47 (19.3)	97 (20.1)	1.00	
9.7 - 10.6	47 (19.3)	97 (20.1)	1.00 (0.60-1.66)	$p = 0.975$
9.0 - 9.7	46 (18.9)	97 (20.1)	0.98 (0.59-1.62)	$p(t) = 0.566$
7.8 - 8.9	51 (21.0)	96 (19.9)	1.09 (0.68-1.76)	
4.8 - 7.7	52 (21.4)	96 (19.9)	1.12 (0.68-1.85)	
Retinol-equivalents (μg):				
2177.9 - 10364.4	50 (20.6)	97 (20.1)	1.00	
1148.4 – 2177.8	55 (22.6)	97 (20.1)	1.08 (0.68-1.73)	$p = 0.928$
921.6 - 1148.3	45 (18.5)	96 (19.9)	0.91 (0.56-1.49)	$p(t) = 0.552$
725.0 - 921.5	45 (18.5)	97 (20.1)	0.89 (0.54-1.47)	
137.8 - 725.0	48 (19.8)	96 (19.9)	0.94 (0.57-1.54)	
Vitamin B_6 (mg):				
2.6 – 4.2	44 (18.1)	97 (20.1)	1.00	
2.3 – 2.5	49 (20.2)	97 (20.1)	1.12 (0.69-1.84)	$p = 0.665$
2.1 – 2.2	41 (16.9)	97 (20.1)	0.95 (0.58-1.56)	$p(t) = 0.286$
1.8 – 2.0	55 (23.6)	96 (19.9)	1.31 (0.79-2.15)	
0.8 – 1.7	54 (22.2)	96 (19.9)	1.26 (0.76-2.10)	
Folic acid (μg):				
368.1 – 631.6	37 (15.2)	97 (20.1)	1.00	
326.4 – 368.0	45 (18.5)	97 (20.1)	1.24 (0.73-2.10)	$p = 0.447$
286.6 – 326.3	56 (23.1)	96 (19.9)	1.54 (0.93-2.55)	$p(t) = 0.152$
246.7 – 286.5	55 (22.6)	97 (20.1)	1.51 (0.91-2.50)	
112.0 – 246.6	50 (20.6)	96 (19.9)	1.40 (0.83-2.36)	
Vitamin C (mg):				
176.9 – 461.9	34 (14.0)	97 (20.1)	1.00	
129.7- 176.8	45 (18.5)	97 (20.1)	1.38 (0.80-2.36)	$p = 0.194$
97.1 – 129.6	50 (20.6)	97 (20.1)	1.50 (0.90-2.50)	$p(t) = 0.010$
67.0 - 97.0	54 (22.2)	96 (19.9)	1.65 (0.97-2.79)	
5.2 – 66.9	60 (24.7)	96 (19.9)	1.82 (1.09-3.12)	
Vitamin E (mg):				
14.4 – 30.2	46 (18.9)	97 (20.1)	1.00	
9.2 – 14.3	55 (22.6)	97 (20.1)	1.20 (0.74-1.96)	$p = 0.667$
7.1 – 9.1	55 (22.6)	97 (20.1)	1.20 (0.73-1.96)	$p(t) = 0.440$
5.4 – 7.0	46 (18.9)	95 (19.7)	0.98 (0.60-1.63)	
1.7 – 5.3	41 (16.9)	97 (20.1)	0.85 (0.50-1.47)	

[1] Derived from the residuals from the regression model of micronutrient intake and total energy intake, and the predicted micronutrient value for the mean energy intake (see Chapter 4) $p(t) = p$ value for trend

Table 6.3.6: Effect of daily micronutrient intake from food/supplements in the 2m before rash onset on zoster risk in individuals without conditions associated with micronutrient deficiency[2] (n=671)

Nutrient-adjusted[1] micronutrient intake	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
Iron (mg):				
13.8 - 240.5	53 (21.8)	97 (20.1)	1.00	
12.0 – 13.7	52 (21.4)	97 (20.1)	1.00 (0.61-1.64)	$p = 0.366$
10.8 - 11.9	35 (14.4)	97 (20.1)	0.66 (0.39-1.12)	$p(t) = 0.903$
9.6 - 10.7	47 (19.3)	97 (20.1)	0.90 (0.54-1.47)	
5.6 - 9.5	56 (23.1)	95 (19.6)	1.09 (0.67-1.79)	
Zinc (mg):				
11.1 – 37.3	49 (20.2)	97 (20.1)	1.00	
10.0 - 11.0	48 (19.8)	97 (20.1)	0.98 (0.60-1.60)	$p = 0.982$
9.0 – 9.9	45 (18.5)	97 (20.1)	0.92 (0.56-1.50)	$p(t) = 0.755$
7.9 – 8.9	49 (20.2)	96 (19.9)	1.01 (0.62-1.64)	
4.7 - 7.8	52 (21.4)	96 (19.9)	1.08 (0.66-1.76)	
Retinol-equivalents (μg):				
2382.7 – 10358.4	52 (21.4)	97 (20.1)	1.00	
1518.6 – 2382.6	46 (18.9)	97 (20.1)	0.86 (0.52-1.43)	$p = 0.462$
1018.0 - 1518.5	54 (22.2)	97 (20.1)	1.01 (0.63-1.64)	$p(t) = 0.864$
777.1 – 1017.9	36 (14.8)	96 (19.9)	0.68 (0.40-1.15)	
137.8 - 777.0	55 (22.6)	96 (19.9)	1.03 (0.63-1.68)	
Vitamin B_6 (mg):				
3.0 – 308.4	52 (21.4)	97 (20.1)	1.00	
2.5 – 2.9	40 (16.5)	97 (20.1)	0.76 (0.46-1.27)	$p = 0.822$
2.1 - 2.4	50 (20.6)	97 (20.1)	0.96 (0.59-1.55)	$p(t) = 0.703$
1.8 - 2.0	50 (20.6)	97 (20.1)	0.97 (0.60-1.56)	
0.7 - 1.8	51 (21.0)	95 (19.6)	1.00 (0.59-1.69)	
Folic acid (μg):				
406.1 – 1092.9	47 (19.3)	97 (20.1)	1.00	
335.8 – 406.0	54 (22.2)	97 (20.1)	1.18 (0.72-1.93)	$p = 0.901$
297.8 – 335.7	48 (19.8)	96 (19.9)	1.03 (0.63-1.70)	$p(t) = 0.700$
251.3 – 297.7	51 (21.0)	97 (20.1)	1.11 (0.67-1.83)	
118.7 – 251.2	43 (17.7)	96 (19.9)	0.92 (0.55-1.55)	
Vitamin C (mg):				
209.1 - 1426.0	42 (17.3)	97 (20.1)	1.00	
147.3 - 209.0	42 (17.3)	97 (20.1)	1.02 (0.61-1.68)	$p = 0.668$
105.9 – 147.2	52 (21.4)	97 (20.1)	1.29 (0.78-2.13)	$p(t) = 0.160$
71.0 – 105.8	52 (21.4)	96 (19.9)	1.28 (0.77-2.13)	
12.0 – 70.9	55 (22.6)	96 (19.9)	1.35 (0.81-2.25)	
Vitamin E (mg):				
18.8 – 531.4	51 (20.6)	97 (20.1)	1.00	
12.1 - 18.7	54 (22.2)	97 (20.1)	1.06 (0.66-1.71)	$p = 0.454$
8.0 – 12.0	52 (21.4)	97 (20.1)	1.03 (0.63-1.66)	$p(t) = 0.221$
5.6 - 7.9	52 (21.4)	96 (19.9)	1.03 (0.63-1.65)	
1.9 - 5.5	35 (14.4)	96 (19.9)	0.68 (0.40-1.16)	

[1] See footnote to Table 6.3.1 [2] See text $p(t) = p$ value for trend

Table 6.3.7: Univariable analyses of the effect of combined daily micronutrient intake in the two months before rash on risk of zoster (n=726)

	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
a) Intakes from foods				
Total micronutrient score[1]*:*				
27 – 35	45 (18.5)	102 (21.1)	1.00	
23 – 26	49 (20.2)	101 (20.9)	1.09 (0.66-1.80)	$p = 0.856$
20 – 22	44 (18.1)	89 (18.4)	1.12 (0.68-1.84)	
16 – 19	54 (22.2)	92 (19.0)	1.31 (0.81-2.12)	
7 – 15	51 (21.0)	99 (20.5)	1.17 (0.71-1.94)	
No. of micronutrients at highest level[2]*:*				
3 - 7	44 (18.1)	98 (20.3)	1.00	
2	42 (17.3)	81 (16.8)	1.16 (0.69-1.92)	$p = 0.856$
1	68 (28.0)	140 (29.0)	1.09 (0.69-1.73)	
0	89 (36.6)	164 (33.9)	1.20 (0.78-1.86)	
b) Intakes from foods & supplements:				
Total micronutrient score[1]*:*				
27 – 35	52 (21.4)	110 (22.8)	1.00	
23 – 26	42 (17.3)	92 (19.1)	0.97 (0.59-1.59)	$p = 0.846$
20 – 22	42 (17.3)	70 (14.5)	1.28 (0.77-2.13)	
16 – 19	55 (22.6)	104 (21.5)	1.11 (0.70-1.77)	
7 – 15	52 (21.4)	107 (22.1)	1.03 (0.63-1.67)	
No. of micronutrients at highest level[2]*:*				
3 - 7	45 (18.5)	93 (19.2)	1.00	
2	43 (17.7)	72 (14.9)	1.23 (0.73-2.07)	$p = 0.632$
1	57 (23.5)	131 (27.1)	0.90 (0.55-1.45)	
0	98 (40.3)	187 (38.7)	1.08 (0.69-1.68)	

[1]Sum of quintile scores of each micronutrient of interest (see text)
[2] At highest quintile of exposure (see text)
$p(t) = p$ value for trend

Table 6.3.8: Univariable analyses of the effect of dietary fruit and vegetable intake in the 2m before rash onset on the risk of zoster (n=726)

Average intake in the last year	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
***Combined** fresh/frozen fruit & vegetables:*				
≥ 8 portions per day	33 (13.6)	105 (21.7)	1.00	
6 - 7 portions per day	43 (17.7)	109 (22.6)	1.38 (0.81-2.35)	$p = 0.010$
4 - 5 portions per day	91 (37.5)	146 (30.2)	2.18 (1.32-3.60)	$p(t)=0.002$
2 - 3 portions per day	61 (25.1)	98 (20.3)	2.13 (1.26-3.61)	
≤ 1 portion per day	15 (6.2)	25 (5.2)	2.11 (0.97-4.57)	
Fresh fruit:				
> 3 portions per day	39 (16.1)	116 (24.0)	1.00	
2 - 3 portions per day	77 (31.7)	157 (32.5)	1.50 (0.95-2.37)	$p = 0.069$
5 - 7 portions per week	67 (27.6)	117 (24.2)	1.70 (1.06-2.72)	$p(t)=0.006$
1 - 4 portions per week	42 (17.3)	70 (14.5)	1.88 (1.08-3.26)	
< 1 portion per week	18 (7.4)	23 (4.8)	2.36 (1.14-4.87)	
Fresh or frozen (all colour) vegetables:				
≥ 5 portions per day	37 (15.2)	104 (21.5)	1.00	
4 portions per day	45 (18.5)	96 (19.9)	1.35 (0.80-2.26)	$p = 0.173$
3 portions per day	60 (24.7)	118 (24.4)	1.49 (0.89-2.49)	$p(t)=0.015$
1 - 2 portions per day	86 (35.4)	140 (29.0)	1.80 (1.12-2.92)	
< 1 portion per day	15 (6.2)	25 (5.2)	1.78 (0.81-23.89)	
*Fresh/ frozen **green, red or yellow** vegetables:*				
≥ 5 portions per day	4 (1.6)	28 (5.9)	1.00	
4 portions per day	17 (7.0)	38 (7.9)	3.27 (0.98-10.86)	$p = 0.047$
3 portions per day	46 (18.9)	103 (21.3)	3.20 (1.05-9.75)	$p(t)=0.027$
1 - 2 portions per day	139 (57.2)	250 (51.8)	4.13 (1.39-12.30)	
< 1 portion per day	37 (15.2)	64 (12.2)	4.02 (1.30-12.46)	

$p(t) = p$ value for trend

Table 6.3.9: Univariable analyses of the effect of anthropometric indices on the risk of zoster

Exposure	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
Body mass index (n=677):				
'Underweight' (≤20)	98 (42.4)	183 (41.0)	0.77 (0.38-1.56)	
'Average' (>20 - 25)	88 (38.1)	155 (34.8)	1.00	$p = 0.523$
'Overweight' (>25 - 30)	31 (13.4)	75 (16.8)	1.09 (0.75-1.57)	
'Obese' (>30)	14 (6.1)	33 (7.4)	0.77 (0.47-1.26)	
*Mindex **(women only: n=379)**[1]:*				
54.03 - 76.91	26 (20.0)	47 (18.9)	0.98 (0.48-2.02)	
76.92 - 84.56	30 (23.1)	51 (20.5)	1.00	$p = 0.861$
84.57 - 92.55	27 (20.8)	51 (20.5)	0.94 (0.47-1.86)	
92.56 - 106.48	27 (20.8)	50 (20.1)	0.95 (0.48-1.90)	
106.49 - 148.77	20 (15.4)	50 (20.1)	0.70 (0.35-1.40)	
*Demiquet **(men only: n=299)**:*				
70.08 - 102.56	22 (21.6)	39 (19.8)	0.96 (0.46-2.01)	
102.57 - 113.72	23 (22.6)	38 (19.3)	1.00	$p = 0.807$
113.73 - 122.50	20 (19.6)	41 (20.8)	0.82 (0.39-1.75)	
122.51 - 133.86	23 (20.8)	41 (20.8)	0.94 (0.45-1.97)	
133.87 - 212.08	14 (13.7)	38 (19.3)	0.63 (0.29-1.40)	

Table 6.3.10: Effects of fresh/frozen fruit and vegetable intake in the last year on the risk of zoster, adjusted for dietary vitamin C intake (n=726)

Average intake in the last year	Univariable OR (95% CI)	Adjusted for dietary vitamin C intake	Adjusted for dietary vit.C intake & smoking
Combined fresh/frozen fruit & vegetables:			
≥ 8 portions per day	1.00	1.00	1.00
6 - 7 portions per day	1.76 (1.02-3.04)	1.71 (0.96-3.05)	1.82 (1.01-3.28)
4 - 5 portions per day	2.25 (1.34-3.79)	2.22 (1.23-4.00)	2.30 (1.26-4.18)
2 - 3 portions per day	2.63 (1.51-4.60)	2.57 (1.31-5.04)	2.73 (1.38-5.41)
≤ 1 portion per day	2.76 (1.23-6.00)	2.67 (1.07-6.69)	2.94 (1.16-7.46)
	p=0.005 *p*(t)=0.0004	*p*=0.06 *p*(t)=0.007	*p*=0.005 *p*(t)=0.005
Fresh fruit:			
> 3 portions per day	1.00	1.00	1.00
2 - 3 portions per day	1.64 (1.03-2.60)	1.54 (0.94-2.54)	1.63 (0.98-2.71)
5 - 7 portions per week	1.77 (1.11-2.82)	1.65 (0.97-2.83)	1.76 (1.02-3.03)
1 - 4 portions per week	2.21 (1.26-3.87)	2.06 (1.05-4.04)	2.27 (1.14-4.51)
< 1 portion per week	2.53 (1.22-5.22)	2.33 (0.98-5.51)	2.84 (1.17-6.92)
	p=0.025 *p*(t)=0.002	*p*=0.240 *p*(t)=0.035	*p*=0.128 *p*(t)=0.015
*Fresh/ frozen **(all colour)** vegetables:*			
≥ 5 portions per day	1.00	1.00	1.00
4 portions per day	1.10 (0.65-1.87)	1.10 (0.58-1.73)	1.31 (0.89-2.64)
3 portions per day	1.45 (0.87-2.42)	1.32 (0.77-2.25)	1.49 (0.86-2.59)
1 - 2 portions per day	1.71 (1.06-2.76)	1.49 (0.89-2.51)	1.82 (0.87-2.72)
< 1 portion per day	2.09 (1.00-4.34)	1.74 (0.78-3.90)	1.69 (0.94-3.06)
	p=0.094 *p* (t)=0.005	*p*=0.371 *p* (t)=0.049	*p*=0.372 *p* (t)=0.048
Fresh or frozen red/yellow/green vegetables:			
≥ 5 portions per day	1.00	1.00	1.00
4 portions per day	2.64 (0.79-8.82)	2.36 (0.70-7.99)	2.35 (0.69-8.02)
3 portions per day	3.51 (1.15-10.74)	2.98 (0.95-9.38)	2.96 (0.93-9.37)
1 - 2 portions per day	3.66 (1.23-10.92)	3.01 (0.97-9.32)	2.90 (0.93-9.04)
< 1 portion per day	4.39 (1.40-13.74)	3.26 (0.97-9.32)	3.23 (0.96-10.89)
	p=0.055 *p* (t)=0.011	*p*=0.291 *p* (t)=0.106	*p*=0.320 *p* (t)=0.129

Table 6.3.11: Effects of nutrient-adjusted vitamin C intake in the last year from foods on risk of zoster, adjusted for fresh/frozen fruit and vegetable intake (n=726)

Nutrient-adjusted vit C intake (mg):	Univariable OR (95% CI)	Adjusted for fruit & vegetable intake	Adjusted for fruit intake	Adjusted for vegetable intake
175.7 - 384.9	1.00	1.00	1.00	1.00
130.1- 177.4	1.49 (0.88-2.53)	1.20 (0.68-2.09)	1.29 (0.75-2.24)	1.46 (0.85-2.50)
97.3 - 130.0	1.56 (0.92-2.65)	1.09 (0.61-1.95)	1.21 (0.68-2.16)	1.47 (0.85-2.50)
68.5 - 97.2	1.60 (0.95-2.70)	1.01 (0.55-1.84)	1.15 (0.64-2.09)	1.42 (0.81-2.47)
5.2 - 68.4	1.95 (1.15-3.30)	1.11 (0.57-2.18)	1.22 (0.64-2.36)	1.56 (0.87-2.79)
	p=0.160 *p (t)*=0.002	*p*=0.640 *p (t)*=0.986	*p*=0.922 *p(t)*=0.767	*p*=0.573 *p (t)*=0.223

Table 6.3.12 Effect* of fresh/frozen vegetable intake in the last 2m on risk of zoster, by age

	Age < 60	Age ≥ 60
Fresh/frozen vegetable intake:		
≥ 5 portions per day	1.00	1.00
4 portions per day	0.94 (0.49-1.81)	2.45 (0.90-6.68)
3 portions per day	1.30 (0.67-2.55)	1.83 (0.69-4.84)
1 - 2 portions per day	0.87 (0.46-1.66)	3.56 (1.40-9.02)
< 1 portion per day	0.73 (0.21-2.58)	3.16 (0.93-10.73)
	p=0.036	

*Adjusted for smoking and fresh fruit intake

Table 6.3.13: Effect of total micronutrient score and number of micronutrients at highest intake level (from food) in the last year on risk of zoster, by age

	Age < 60 years (n=390)	Age ≥ 60 years (n=336)
Micronutrient score:		
27 – 35	1.00	1.00
23 – 26	0.71 (0.37-1.36)	2.03 (0.83-4.93)
20 – 22	0.79 (0.41-1.55)	3.12 (1.35-7.20)
16 - 19	0.87 (0.47-1.60)	2.80 (1.21-6.47)
7 – 15	0.45 (0.21-0.95)	3.73 (1.64-8.50)
	p = 0.281 *p (trend)* = 0.131	p = 0.013 *p (trend)* = 0.002
No. of micronutrients at highest intake level:		
3 – 7	1.00	1.00
2	1.66 (0.87-3.17)	1.06 (0.44-2.53)
1	0.71 (0.39-1.31)	2.59 (1.19-5.61)
0	0.96 (0.54-1.71)	2.60 (1.23-5.50)
	p = 0.061	p = 0.007 *p (trend)* = 0.002

Table 6.4.1: Univariable effects of cumulative UVR exposure and intensity of UVR exposure from sunlight in childhood on the risk of zoster

UVR exposure (MED)	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
Cumulative UVR exposures in childhood				
Total UVR exposure in warmer months from non-holidays+ holidays combined (n=616):				
246.5 - 504.0	29 (13.5)	78 (19.4)	1.00	
504.1 - 630.2	49 (22.9)	81 (21.1)	1.59 (0.90-2.79)	$p = 0.401$
630.3 - 730.3	41 (19.2)	82 (20.4)	1.33 (0.75-2.35)	$p(t) = 0.148$
730.4 - 936.9	47 (22.1)	81 (20.0)	1.62 (0.91-2.88)	
937.0 - 2448.1	48 (22.4)	80 (19.9)	1.66 (0.94-2.94)	
Total weekly UVR exposure in warmer months, excluding holidays (n=631):				
3.03 - 16.20	22 (10.1)	82 (19.8)	1.00	
16.21 - 20.51	53 (24.3)	82 (19.8)	2.54 (1.39-4.64)	$p = 0.006$
20.52 - 23.92	59 (27.1)	86 (20.8)	2.58 (1.44-4.63)	$p(q) = 0.002$
23.93 - 27.35	47 (21.6)	80 (19.4)	2.31 (1.25-4.26)	$p(t) = 0.320$
27.35 - 84.21	37 (17.0)	83 (20.1)	1.63 (0.88-3.02)	
Total UVR exposure on summer holidays (n=689):				
No holidays	89 (38.2)	181 (39.7)	2.38 (1.16-4.87)	
2.80 - 39.2	10 (4.3)	51 (11.2)	1.00	$p = 0.02$
39.3 – 61.1	33 (14.2)	57 (12.5)	2.86 (1.28-6.37)	$p(t) = 0.043$[1]
61.2 - 84.6	26 (11.2)	54 (11.8)	2.33 (1.02- 5.32)	
84.7 – 160.2	41 (17.6)	58 (12.7)	3.72 (1.68-8.27)	
160.3 – 1037.2	34 (14.6)	55 (12.1)	3.17 (1.41-7.13)	
Intensity of UVR exposure in childhood				
Highest daily UVR exposure per week in warmer months, excluding holidays (n=631):				
0.43 - 1.62	29 (13.3)	82 (19.9)	1.00	
1.63 - 2.16	50 (22.9)	77 (18.6)	1.83 (1.06-3.17)	$p = 0.098$
2.17 - 3.33	42 (19.3)	73 (17.7)	1.68 (0.94-2.98)	$p(t) = 0.643$
3.34 - 4.32	55 (25.2)	85 (20.6)	1.84 (1.06-3.17)	
4.33 - 15.27	42 (19.3)	96 (23.2)	1.21 (0.69-2.12)	
Daily UVR exposure on holiday (n=689):				
No holidays/trips	87 (38.2)	178 (39.0)	1.43 (0.79-2.86)	
0.40 - 4.32	18 (7.6)	54 (12.0)	1.00	
4.32 - 5.19	28 (11.8)	58 (12.7)	1.44 (0.72-2.86)	$p = 0.093$
5.19 - 6.05	23 (9.7)	49 (10.8)	1.35 (0.67-2.72)	$p(t) = 0.049$[1]
6.05 - 6.91	26 (21.4)	55 (12.1)	1.45 (0.71-3.00)	
6.91 - 15.27	51 (11.3)	62 (13.6)	2.53 (1.31-4.91)	

[1]Using holidaymakers only (n=295) $p(t) = p$ value for trend $p(q) = p$ value for quadratic association

Table 6.4.2: Univariable effects of intermittent UVR exposure from sunlight in childhood on risk of zoster

UVR exposure (MED)	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
Intermittency in non-holiday periods				
Ratio of non-schoolday to schoolday exposure in warmer months (n=631)[1]:				
≤ 1.00	13 (6.0)	24 (5.8)	1.00	
1.1 - 1.8	45 (20.6)	97 (23.5)	0.89 (0.41-1.92)	$p = 0.741$
1.9 - 2.3	46 (21.1)	96 (23.2)	0.86 (0.39-1.87)	$p(t) = 0.266$
2.4 - 2.9	55 (25.2)	98 (23.7)	1.06 (0.49-2.30)	
3.0 - 9.0	59 (27.1)	98 (23.7)	1.17 (0.54-2.52)	
Intermittency due to childhood holidays				
Ratio of holiday to non-holiday UVR exposure in warmer months (n=616)[2]:				
≤ 1.00 (including no holidays)	106 (49.5)	229 (57.0)	1.00	
1.01 - 1.20	31 (14.5)	43 (10.7)	1.60 (0.93-2.75)	$p = 0.233$
1.11 - 1.42	33 (15.4)	44 (10.9)	1.68 (1.00-2.84)	$p(t) = 0.414$
1.43 - 1.85	24 (11.2)	44 (10.9)	1.17 (0.68-2.00)	
1.86 - 6.0	20 (9.4)	42 (10.5)	1.11 (0.61-2.20)	

[1] Highest daily exposure on schoolday: highest daily exposure on non-schoolday in summer months
[2]Highest daily exposure on holiday: highest daily exposure on non-holiday in summer months
$p(t) = p$ value for trend $p(q) = p$ value for quadratic association

Table 6.4.3: Univariable effects of cumulative UVR exposure from sunlight in the last year on risk of zoster (n=729)

UVR exposure (MED)	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
Total UVR exposure in warmer months, from non-holidays & holidays combined:				
0 - 157.7	37 (15.2)	92 (19.0)	1.00	
157.8 - 245.7	54 (22.1)	102 (21.0)	1.35 (0.81-2.24)	$p = 0.290$
245.8 - 363.6	42 (17.2)	96 (19.8)	1.15 (0.66-2.01)	$p(t) = 0.073$
363.7 - 534.2	49 (20.1)	98 (20.2)	1.32 (0.77-2.27)	
534.3 - 1169.3	62 (25.4)	97 (20.0)	1.76 (1.02-3.02)	
Total weekly UVR exposure in warmer months, excluding holidays:				
0 - 4.7	43 (17.6)	96 (19.8)	1.00	
4.8 - 8.4	45 (18.4)	97 (20.0)	1.04 (0.62-1.73)	$p = 0.311$
8.5 - 12.1	54 (22.1)	104 (21.4)	1.20 (0.72-1.99)	$p(t) = 0.120$
12.2 - 18.1	41 (16.8)	95 (19.6)	1.00 (0.58-1.72)	
18.2 - 43.2	61 (25.0)	93 (19.2)	1.61 (0.95-2.74)	
Total UVR exposure on summer holidays/trips:				
No holidays	107 (43.9)	193 (39.8)	1.67 (0.93-2.99)	
0 - 22.9	20 (8.2)	58 (12.0)	1.00	$p = 0.224$
23.0 – 41.0	24 (9.8)	59 (12.1)	1.19 (0.59-2.41)	$p(t) = 0.926$[1]
41.1 - 70.1	34 (13.9)	58 (12.0)	1.72 (0.88-3.37)	$p(q) = 0.035$[1]
70.2 – 132.7	37 (15.2)	59 (12.1)	1.88 (0.95-3.71)	
132.8 – 893.4	22 (9.0)	58 (12.0)	1.10 (0.53-2.29)	
Total UVR exposure on winter holidays/trips:				
No holidays	159 (65.2)	350 (72.1)	0.71 (0.43-1.16)	
0 – 4.9	30 (12.3)	45 (9.3)	1.00	$p = 0.289$
5.0 – 22.9	25 (10.2)	45 (9.3)	0.87 (0.44-1.69)	$p(t) = 0.452$[1]
23.0 – 1186.7	30 (12.3)	45 (9.3)	1.03 (0.54-2.00)	
Total UVR exposure during holidays/trips (summer & winter combined):				
No holidays	83 (34.0)	160 (33.0)	1.08 (0.65-1.81)	
0 - 22.8	32 (13.1)	66 (13.6)	1.00	$p = 0.642$
22.9 - 42.6	23 (9.4)	64 (13.2)	0.73 (0.38-1.39)	$p(t) = 0.841$[1]
42.7 - 70.7	37 (15.2)	65 (13.4)	1.18 (0.66-2.12)	
70.8 - 140.8	38 (15.6)	65 (13.4)	1.24 (0.66-2.25)	
140.9 - 1186.8	31 (12.7)	65 (13.4)	0.98 (0.52-1.81)	
Total UVR exposure from sunbathing during non-holiday periods:				
None	135 (55.3)	283 (58.4)	1.00	
0.75 - 450.2	36 (14.8)	67 (13.8)	1.14 (0.72-1.80)	$p=0.862$
450.3 - 1618.1	36 (14.8)	67 (13.6)	1.16 (0.73-1.84)	
1618.2 - 8640.6	37 (15.1)	69 (14.2)	1.15 (0.72-1.83)	

[1] Using holidaymakers only $p(t) = p$ value for trend $p(q) = p$ value for quadratic association

Table 6.4.4: Univariable effects of intensity and intermittency of UVR exposure from sunlight last year on risk of zoster (n=729)

UVR exposure (MED)	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
Intensity of UVR exposure				
Highest daily UVR exposure per week in warmer months, excluding holidays:				
0 - 1.0	50 (20.5)	97 (20.0)	1.00	
1.1 - 1.6	45 (18.4)	120 (24.7)	0.74 (0.45-1.22)	$p = 0.226$
1.7 - 2.9	44 (18.0)	87 (17.9)	1.02 (0.60-1.73)	$p(t) = 0.261$
3.0 - 3.8	70 (28.7)	109 (22.5)	1.29 (0.78-2.14)	
3.9 - 11.5	35 (14.3)	72 (14.9)	1.00 (0.56-1.79)	
Daily UVR exposure on summer/winter holidays:				
No holidays/trips	83 (33.9)	161 (33.1)	1.03 (0.62-1.69)	
0 - 2.2	33 (13.5)	66 (13.6)	1.00	$p = 0.152$
2.3 - 3.8	21 (8.6)	65 (13.4)	0.63 (0.33-1.20)	$p(t) = 0.722$[1]
3.9 - 5.5	47 (19.2)	65 (13.4)	1.49 (0.83-2.65)	
5.6 - 7.6	27 (11.2)	65 (13.4)	0.84 (0.45-1.59)	
7.7 - 15.2	34 (13.9)	65 (13.4)	1.05 (0.58-1.92)	
Intermittency of exposure				
Ratio of non-work day:work day exposure in summer months[1]*:*				
≤ 1.00*	151 (61.9)	292 (60.2)	1.00	
1.1 - 3.0	32 (13.1)	50 (10.3)	1.22 (0.70-2.12)	$p = 0.410$
3.1 – 8.0	29 (11.9)	65 (13.4)	0.79 (0.46-1.36)	$p(t) = 0.131$
8.1 - 16.0	18 (7.4)	36 (7.2)	0.89 (0.46-1.72)	
16.1 - ∞	14 (5.7)	42 (8.7)	0.60 (0.31-1.19)	
Ratio of holiday to non-holiday UVR exposure in summer months[2]*:*				
≤ 1.00 (including no holidays)	125 (51.2)	240 (49.5)	1.00	
0.75 - 1.64	24 (9.8)	60 (12.4)	0.75 (0.44-1.28)	$p = 0.559$
1.65 - 2.40	40 (16.4)	62 (12.8)	1.22 (0.77-1.92)	$p(t) = 0.661$
2.41 - 3.64	27 (11.1)	62 (12.8)	0.84 (0.50-1.39)	
3.65 - 26.64	28 (11.5)	61 (12.6)	0.90 (0.54-1.48)	
Ratio of holiday to non-holiday UVR exposure in winter months[2]*:*				
≤ 1.00 (including no holidays)	169 (69.3)	366 (75.5)	1.00	
1.01 - 4.9	22 (9.0)	39 (8.0)	1.21 (0.70-2.09)	$p = 0.328$
5.0 - 15.1	26 (10.7)	41 (8.1)	1.35 (0.80-2.27)	$p(t) = 0.065$
15.2 - ∞	27 (11.1)	39 (8.0)	1.54 (0.90-2.63)	

* Includes individuals who did not work or worked 7 days/week

[1] Highest daily exposure on non-work day: highest daily exposure on workday in summer months. Ratios were reversed for individuals who worked for less than half the week (see text)

[2] Highest daily exposure on holiday: highest daily exposure on non-holiday (see text)

$p(t) = p$ value for trend $p(q) = p$ value for quadratic association

Table 6.4.5: Univariable effects of cumulative, intermittency and intensity of UVR exposure from sunlight in the month before rash onset on the risk of zoster (n=729)

UVR exposure MED)	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
Cumulative UVR exposures				
Total UVR exposure (non-holidays+ holidays):				
0 - 3.06	39 (16.0)	97 (20.0)	1.00	
3.06 - 11.40	56 (23.0)	98 (20.2)	1.59 (0.92-2.77)	$p = 0.356$
11.40 - 33.33	44 (18.0)	97 (20.0)	1.44 (0.70-2.98)	$p(t) = 0.102$
33.33 - 64.02	51 (20.9)	96 (19.8)	1.83 (0.85-3.92)	
64.02 - 293.46	54 (22.1)	97 (20.0)	1.99 (0.91-4.36)	
Total UVR exposure on holidays & trips:				
No holidays	199 (81.6)	421 (86.8)	1.00	
0 - 26.39	24 (10.2)	32 (6.6)	1.56 (0.90-2.72)	$p = 0.181$
26.39 - 266.29	21 (8.6)	32 (6.6)	1.41 (0.80-2.51)	$p(t) = 0.107$
Total non-holiday UVR exposure from sunbathing:				
None	203 (82.9)	378 (78.0)	1.00	
0.75 - 8.8	24 (9.8)	54 (11.1)	0.81 (0.47-1.39)	p=0.146
8.9 - 171.05	17 (7.0)	53 (10.9)	0.57 (0.32-1.04)	$p(t)$=0.051
Intensity of UVR exposure				
Highest daily non-holiday UVR exposure:				
0 - 0.16	37 (15.2)	98 (20.2)	1.00	
0.17 - 0.80	57 (23.4)	96 (19.8)	1.80 (1.03-3.14)	$p = 0.164$
0.81 - 1.81	57 (23.4)	105 (21.6)	1.92 (0.92-3.99)	$p(t) = 0.165$
1.82 - 3.25	39 (16.0)	93 (19.2)	1.56 (0.68-3.53)	
3.26 - 8.15	54 (22.1)	93 (19.2)	2.20 (0.98-4.96)	
Highest daily UVR exposure on holidays & trips:				
No holidays	199 (81.6)	421 (86.8)	1.00	
0 - 3.4	20 (8.2)	32 (6.6)	1.29 (0.72-2.31)	$p = 0.146$
3.4 - 14.4	25 (10.2)	32 (6.6)	1.71 (0.97-3.00)	$p(t) = 0.05$
Intermittency of UVR exposure				
Ratio of holiday to non-holiday exposure[2]*:*				
≤ 1.00 (including no holidays)	209 (85.7)	436 (89.9)	1.00	
1.01 - 2.50	24 (9.8)	24 (5.0)	2.17 (1.16-4.07)	$p = 0.048$
2.51 - 578.1	11 (4.5)	25 (5.1)	0.92 (0.44-1.90)	$p(t) = 0.362$
Sunburns causing severe erythema/blistering:				
No	236 (96.7)	474 (97.7)	1.00	
Yes	8 (3.3)	11 (2.3)	1.43 (0.56-3.67)	$p = 0.461$

*, [1, 2] See footnotes to Table 6.4.3 & Table 6.4.4 $p(t) = p$ value for trend

Table 6.4.6: Univariable analyses of effect of skin response to UVR exposure on the risk of zoster

Skin response to UVR exposure	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
Response to initial exposure – propensity to burn (n=677):				
Tans only	86 (37.4)	175 (39.1)	1.00	
Tans + burns	68 (29.6)	157 (35.1)	0.89 (0.50-1.32)	$p = 0.252$
Burns + peels	69 (30.0)	104 (23.3)	1.37 (0.90-2.09)	$p(t) = 0.175$
Blisters + peels	7 (3.0)	11·(2.5)	1.33 (0.60-1.32)	
Response to chronic exposure – ability to tan (n=709):				
Deep tan	58 (24.4)	133 (28.2)	1.00	
Moderate tan	115 (48.3)	218 (46.3)	1.23 (0.84-1.81)	$p = 0.153$
Mild tan + peeling	43 (18.1)	95 (20.2)	1.04 (0.64-1.70)	$p(t) = 0.162$
Freckling without tan	22 (9.2)	25 (5.3)	2.20 (1.08-4.50)	

Table 6.4.7: Independent effects of weekly non-holiday UVR exposure in the warmer months and total summer holiday exposure in childhood on the risk of zoster (n=616)[1]

UVR exposure (MED)	Univariable OR	Adjusted for other variable in the Table	Adjusted for other variable + ethnicity
Weekly UVR exposure in warmer months, excluding holidays:			
78.8 – 421.3	1.00	1.00	1.00
421.4 – 533.3	2.66 (1.44-4.92)	2.30 (1.22-4.33)	2.30 (1.22-4.34)
533.4 – 622.1	2.59 (1.43-4.70)	2.40 (1.31-4.41)	2.37 (1.28-4.38)
622.2 – 711.0	2.42 (1.30-4.53)	2.17 (1.14-4.11)	2.16 (1.13-4.11)
711.1 – 2189.2	1.72 (0.92-3.21)	1.58 (0.83-2.99)	1.61 (0.83-3.15)
	p = 0.007 p(q) = 0.002	p = 0.029 p(q) = 0.008	p = 0.041 p(q) = 0.012
Total UVR exposure during summer holidays:			
No holidays	1.91 (0.95-3.88)	1.77 (0.86-3.64)	1.78 (0.87-3.66)
2.80 - 39.2	1.00	1.00	1.00
39.3 – 61.1	2.75 (1.24-6.10)	2.48 (1.10-5.59)	2.45 (1.08-5.54)
61.2 - 84.6	2.23 (0.98-5.07)	1.83 (0.79-4.26)	1.82 (0.78-4.25)
84.7 – 160.2	3.18 (1.44-7.04)	2.70 (1.20-6.10)	2.65 (1.17-5.98)
160.3 – 1037.2	2.74 (1.24-6.08)	2.27 (1.01-5.11)	2.21 (0.98-4.98)
	p=0.043	p=0.157	p=0.188

[1] Individuals with information on all covariates of interest p(q) = p value for quadratic association

Table 6.4.8: Effect of cumulative holiday UVR exposure in childhood on risk of zoster, by age

UVR exposure (MED)	Age < 60yrs[1] (n=344)	Age > 60yrs[1] (n=272)
Cumulative holiday UVR exposure in childhood (n=616):		
No holidays	4.07 (1.29-12.83)	0.76 (0.28-2.07)
2.8 - 39.2	1.00	1.00
39.3 – 61.1	2.78 (0.80-9.70)	1.66 (0.50-5.50)
61.2 - 84.6	4.62 (1.44-16.26)	0.44 (0.11-1.85)
84.7 - 160.2	5.95 (1.79-19.72)	0.82 (0.22-3.11)
160.3 -1037.2	4.00 (1.11-12.44)	1.01 (0.30-3.48)
	p = 0.038 p(q) = 0.027 [(2)]	p = 0.234

[1] Adjusted for weekly non-holiday UVR [2] Using holidaymakers only (n=187)
p(q) = p value for quadratic association

Table 6.4.9: Comparison of effect of total (holiday + non-holiday) cumulative UVR exposure in childhood on risk of zoster amongst all individuals and amongst holidaymakers

UVR exposure (MED)	Amongst holidaymakers and non-holidaymakers (n=616)	Amongst holidaymakers only (n=278)
Total UVR exposure in warmer months of childhood:		
246.5 - 504.0	1.00	1.00
504.1 - 630.2	1.59 (0.90-2.79)	1.30 (0.40-4.26)
630.3 - 730.3	1.33 (0.75-2.35)	1.33 (0.47-3.72)
730.4 - 936.9	1.62 (0.91-2.88)	2.04 (0.71-5.88)
937.0 - 2448.1	1.66 (0.94-2.94)	1.98 (0.69-5.69)
	$p = 0.401$ $p(t) = 0.148$	$p = 0.498$ $p(t) = 0.093$

$p(t) = p$ value for trend

Table 6.4.10: Effect of intermittency of holiday UVR exposure in childhood, at different levels of intensity of baseline (non-holiday) UVR exposure (n=616)

UVR exposure (MED)	Highest daily non-holiday UVR <4 MED (n=216)	Highest daily non-holiday UVR ≥4 MED (n=400)
Ratio of holiday to non-holiday UVR exposure in summer months[1]*:*		
≤ 1.00	1.00	1.00
1.01 - 1.50	1.35 (0.59-3.08)	1.55 (0.97-2.47)
1.50 – 6.00	0.77 (0.40-1.46)	4.07 (1.46-11.30)
	$p = 0.018$	

[2] Highest daily exposure on holiday: highest daily exposure on non-holiday in summer months

Table 6.4.11: Independent effects of total cumulative UVR exposure in the warmer months of last year and in month before rash onset on risk of zoster (n=709[1])

UVR exposure (MED)	Univariable OR	Adjusted for ethnicity, ability to tan and current illness
Total UVR exposure in warmer months of last year:		
0 - 157.7	1.00	1.00
157.8 - 245.7	1.35 (0.80-2.26)	1.49 (0.86-2.56)
245.8 - 363.6	1.19 (0.67-2.11)	1.37 (0.75-2.50)
363.7 - 534.2	1.26 (0.70-2.14)	1.47 (0.82-2.64)
534.3 - 1169.3	1.76 (1.02-3.06)	2.14 (1.19-3.83)
	$p = 0.298$ $p(t) = 0.094$	$p = 0.131$ $p(t) = 0.023$
Total UVR exposure in month before rash onset:		
0 - 3.06	1.00	1.00
3.06 - 11.40	1.70 (0.96-3.00)	1.91 (1.06-3.45)
11.40 - 33.33	1.56 (0.75-3.26)	1.89 (0.88-4.09)
33.33 - 64.02	1.92 (0.88-4.18)	2.44 (1.08-5.51)
64.02 - 293.46	2.09 (0.94-4.64)	2.82 (1.23-6.49)
	$p = 0.311$ $p(t) = 0.099$	$p = 0.112$ $p(t) = 0.024$

[1] Individuals with information on all covariates of interest $p(t) = p$ value for trend

Table 6.4.12: Effect[1] of total (holiday & non-holiday) UVR exposure in the month before rash onset on risk of zoster, by age

Total UVR exposure (MED)	Age < 60yrs (n=390)	Age > 60yrs (n=339)
Total (holiday+non holiday) UVR exposure:		
0 - 3.06	1.00	1.00
3.06 - 11.40	2.40 (0.90-6.37)	1.68 (0.78-3.59)
11.40 - 33.33	1.69 (0.46-6.16)	3.00 (1.08-8.33)
33.33 - 64.02	4.88 (1.29-18.48)	1.07 (0..34-3.37)
64.02 - 293.46	3.88 (0.99-15.14)	1.86 (0.61-5.67)
	$p = 0.016$ $p(t) = 0.015$	$p=0.079$

[1] Adjusted for current illness, ethnicity and ability to tan $p(t) = p$ value for trend

Table 6.4.13: Effect[1] of total holiday UVR exposure in the month before rash onset on risk of zoster, by propensity to burn on initial UVR exposure (n=677)[2]

UVR exposure (MED)	Tans	Peels/Blisters
Total holiday UVR exposure:		
No holidays	1.00	1.00
0 - 26.39	1.09 (0.52-2.26)	3.40 (1.11-10.41)
26.39 - 266.29	1.87 (0.97-3.63)	0.49 (0.10-2.41)
	$p = 0.046$	

[1]Adjusted for ethnicity and current illness [2] Individuals with information on all covariates of interest

Table 6.4.14: Effect of total (holiday+non holiday) UVR exposure in the month before rash onset on risk of zoster in all individuals and in matched sets containing confirmed cases

UVR exposure from holidays+non-holidays in month before rash onset (MED)	Adjusted[1] OR (total study population)	Adjusted[1] OR (matched sets with confirmed cases)
a) Amongst individuals of all ages:	**(n=709)**	**(n=266)**
0 - 3.06	1.00	1.00
3.06 - 11.40	1.91 (1.06-3.45)	4.14 (1.14-15.03)
11.40 - 33.33	1.89 (0.88-4.09)	3.48 (0.79-15.23)
33.33 - 64.02	2.44 (1.08-5.51)	4.28 (0.95-19.34)
64.02 - 293.46	2.82 (1.23-6.49)	6.52 (1.38-30.88)
	$p = 0.112$ $p(t) = 0.024$	$p = 0.096$ $p(t) = 0.033$
b) Amongst individuals aged <60y:	**(n=390)**	**(n=140)**
0 - 3.06	1.00	1.00
3.06 - 11.40	2.40 (0.90-6.37)	2.77 (0.22-33.84)
11.40 - 33.33	1.69 (0.46-6.16)	1.46 (0.08-26.94)
33.33 - 64.02	4.88 (1.29-18.48)	6.25 (0.33-117.97)
64.02 - 293.46	3.88 (0.99-15.14)	5.14 (0.26-101.86)
	$p = 0.016$ $p(t) = 0.015$	$p = 0.155$ $p(t) = 0.059$

[1] Adjusted for ethnicity, ability to tan and current illness $p(t) = p$ value for trend

Table 6.4.15: Summary of adjusted effects of UVR variables on risk of zoster

Exposure	Effect in childhood	Effect in the last year	Effect in month before rash onset
1. Cumulative UVR exposure:			
- Total exposure in warmer months	No effect	***Increased risk?[1]***	***Increased risk?[1]*** ***Effect modified by age?[1]***
- Weekly non-holiday UVR in warmer months	**Strongly increased risk**	No effect	-
- Total holiday UVR	**Strongly increased risk if <60y**	No effect	No effect
2 .Intensity of UVR exposure:			
- Maximum daily non-holiday UVR	***Increased risk?[1]***	No effect	No effect
- Maximum daily holiday UVR	No effect	No effect	***Increased risk?[1]***
3 Intermittency of UVR exposure:			
- During non-holiday periods	No effect	No effect	-
- Due to holidays	**Increased risk if non-holiday UVR >4MED**	***Winter: increased risk?[1]***	***Increased risk (pattern unclear)?[1]***
- Effect of sunburns	(No information)	No effect	No effect
4. Effect of sunbathing	(No information)	No effect	***Protective effect?[1]***
5. Effect of hats and protective clothing	No effect	***Decreased risk with protective clothing for non-holiday exposure?[1]***	-
6. Effect of skin type on UVR exposures	No effect	No effect	***Increased risk from total holiday exposure amongst those with propensity to burn?***
7. Effect of medical UVR exposures/sunbeds	No effect	No effect	No effect

[1] Weakly statistically significant association

Table 6.5.1: Univariable effects of stress in the last 12 months on the risk of zoster (n=726)

Stressful events/feelings in last 12 m	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
All prompted+unprompted events/feelings:				
None	23 (9.5)	81 (16.8)	1.00	
1	53 (21.8)	122 (25.3)	1.62 (0.90-2.90)	$p = 0.012$
2	56 (23.1)	107 (22.2)	1.92 (1.09-3.37)	$p(t) = 0.0003$
3	42 (17.3)	78 (16.1)	2.19 (1.17-4.08)	
4	30 (12.4)	46 (9.5)	2.50 (1.28-4.88)	
≥5	40 (16.0)	49 (10.1)	3.20 (1.64-6.24)	
No. of prompted events:				
None	50 (20.6)	114 (23.6)	1.00	
1	52 (21.4)	133 (27.5)	0.94 (0.59-1.49)	$p = 0.124$
2	63 (25.9)	108 (22.4)	1.46 (0.90-2.38)	$p(t) = 0.014$
3	36 (14.8)	68 (14.1)	1.31 (0.75-2.28)	
4	19 (7.8)	34 (7.0)	1.41 (0.71-2.79)	
≥5	23 (9.3)	26 (5.4)	2.26 (1.12-4.54)	
No of unprompted events:				
None	179 (73.7)	407 (84.3)	1.00	
1	52 (21.4)	64 (13.2)	1.91 (1.25-2.91)	$p = 0.003$
2	12 (4.9)	12 (2.5)	2.17 (0.95-5.00)	$p(t) = 0.0009$
No of unprompted feelings:				
None	178 (73.3)	389 (80.6)	1.00	
1	61 (25.1)	88 (18.2)	1.56 (1.07-2.27)	$p = 0.068$
2	4 (1.6)	6 (1.2)	1.60 (0.45-5.76)	$p(t) = 0.003$
Effect[3] of prompted[1] events				
Death of spouse	1 (0.4)	4 (0.8)	0.50 (0.06-4.47)	$p = 0.509$
Death of close family	19 (7.8)	33 (6.8)	1.17 (0.65-2.10)	$p = 0.612$
Death of close friend	29 (11.9)	42 (8.7)	1.43 (0.87-2.35)	$p = 0.166$
Serious illness - spouse	14 (5.8)	25 (5.2)	1.13 (0.57-2.24)	$p = 0.725$
Serious illness – close family	46 (18.9)	63 (13.0)	1.55 (1.02-2.35)	$p = 0.040$
Serious illness - close friend	12 (4.9)	12 (2.5)	1.94 (0.87-4.34)	$p = 0.107$
Divorce/separation	15 (6.2)	29 (6)	1.04 (0.54-2.00)	$p = 0.911$
Difficulties with family members	75 (30.9)	153 (31.7)	0.96 (0.69-1.34)	$p = 0.822$
Difficulties with neighbours	30 (12.4)	75 (15.5)	0.75 (0.47-1.20)	$p = 0.230$
Serious financial worries	64 (26.3)	113 (23.4)	1.19 (0.82-1.72)	$p = 0.371$
Moving house	25 (10.3)	39 (8.1)	1.33 (0.76-2.33)	$p = 0.313$
Difficulties at work/unemployment	82 (33.7)	162 (33.5)	0.99 (0.67-1.48)	$p = 0.973$
Effect[3] of unprompted[2] events/feelings				
Arguments with spouse/ex-spouse	12 (4.9)	20 (4.1)	1.21 (0.58-2.50)	$p = 0.615$
Accidents/robberies/assaults	12 (4.9)	17 (3.5)	1.43 (0.67-3.04)	$p = 0.357$
Problems with accommodation / builders	19 (7.8)	30 (6.2)	1.30 (0.71-2.34)	$p = 0.400$
Court cases/trouble with the law	7 (2.9)	8 (1.7)	1.83 (0.63-5.29)	$p = 0.278$
Organising events (eg.weddings, parties)	5 (2.1)	1 (0.2)	10.0 (1.17-85.57)	$p = 0.012$
Moved away from family	4 (1.7)	9 (1.9)	0.89 (0.27-2.89)	$p = 0.844$
Continuing bereavement	14 (5.8)	8 (1.7)	3.81 (1.53-9.49)	$p = 0.003$
Concerns about own health	44 (18.1)	77 (15.9)	1.18 (0.78-1.80)	$p = 0.430$
Feeling isolated from family/ friends	9 (3.7)	8 (1.7)	2.39 (0.88-6.49)	$p = 0.086$

[1] Responses to prompted questions [2] Responses to open question [3] Compared to not experiencing the event

Table 6.5.2: Univariable effects of incident stress in the two months before rash onset on the risk of zoster (n=726)

Incident stressful events/feelings	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
All incident events/feelings combined:				
None	153 (63.0)	388 (80.3)	1.00	
1	70 (28.8)	80 (16.6)	2.36 (1.59-3.48)	$p<0.0001$
≥2	20 (8.2)	15 (3.1)	3.50 (1.75-6.98)	$p(t)< 0.0001$
≥1 *incident prompted event (vs not)*	70 (28.8)	80 (16.6)	2.16 (1.46-3.19)	$p = 0.0001$
≥1 *incident unprompted event (vs not)*	27 (11.1)	14 (2.9)	4.05 (2.09-7.87)	$p < 0.0001$
≥1 *incident unprompted feeling (vs not)*	5 (2.1)	4 (0.8)	2.50 (0.67-9.31)	$p=0.172$
No. of incident prompted events:				
None	173 (71.2)	403 (83.4)	1.00	$p =0.0005$
1	60 (24.7)	68 (14.1)	2.16 (1.43-3.25)	$p(t) = 0.0003$
≥2	10 (4.1)	12 (2.5)	2.15 (0.91-5.07)	
Effect[3] of prompted[1] event				
Death spouse/close family/close friend	15 (6.2)	11 (2.3)	2.73 (1.25-5.94)	$p = 0.011$
Serious illness spouse/close family or friend	12 (4.9)	12 (2.5)	1.94 (0.87-4.34)	$p = 0.107$
Divorce/separation	5 (2.1)	6 (1.2)	1.77 (0.50-6.24)	$p = 0.381$
Difficulties with family members	9 (3.7)	20 (4.1)	0.87 (0.41-2.06)	$p = 0.838$
Difficulty with neighbours	6 (2.5)	11 (2.3)	1.09 (0.39-1.95)	$p = 0.737$
Serious financial worries	8 (3.3)	6 (1.2)	2.67 (0.93-7.69)	$p = 0.068$
Moving house	8 (3.3)	4 (0.8)	4.00 (1.20-13.28)	$p = 0.019$
Difficulties at work/unemployment	21 (8.6)	22 (4.5)	2.39 (1.16-4.92)	$p = 0.017$
Effect[3] of unprompted[2] events/feelings				
Arguments with spouse/ex-spouse	3 (1.2)	1 (0.2)	6.00 (0.62-57.68)	$p = 0.088$
Accidents/robberies/assaults	7 (2.9)	4 (0.8)	3.50 (1.02-11.96)	$p = 0.040$
Problems with accommodation / builders	5 (2.1)	5 (1.0)	2.19 (0.58-8.36)	$p = 0.250$
Court cases/trouble with law	4 (1.6)	2 (0.4)	4.00 (0.73-21.84)	$p = 0.096$
Organising weddings, parties etc	2 (0.8)	0	-	
Concerns about own health	5 (1.0)	5 (2.1)	2.00 (0.58-6.91)	$p = 0.278$
Feeling isolated from family and/or friends	1 (0.4)	2 (0.4)	1.00 (0.09-11.03)	$p = 1.000$

[1] Responses to prompted questions [2] Responses to open question [3] Compared to not experiencing the event

Table 6.5.3: Univariable effects of prevalent stress in the two months before rash onset on risk of zoster (n=726)

Prevalent stressful events/feelings	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
All prevalent events/feelings combined:				
None	42 (17.3)	149 (30.9)	1.00	
1	76 (31.3)	160 (33.1)	1.44 (0.99-2.10)	$p = 0.013$
2	62 (25.5)	99 (20.5)	2.05 (1.30-3.23)	
≥3	63 (25.9)	75 (15.5)	1.67 (0.98-2.85)	
≥1 *prevalent prompted event (vs not)*	147 (60.5)	256 (53)	1.35 (0.99-1.85)	$p = 0.059$
≥1 *prevalent unprompted event (vs not)*	25 (10.3)	31 (6.4)	1.72 (0.98-3.03)	$p = 0.062$
≥1 *prevalent unprompted feeling (vs not)*	50 (20.6)	72 (14.9)	1.53 (1.01-2.31)	$p = 0.046$
No. of prevalent prompted events:				
None	96 (39.5)	227 (47.0)	1.00	
1	81 (33.5)	154 (31.9)	1.25 (0.88-1.78)	$p = 0.171$
2	48 (19.8)	69 (14.3)	1.64 (1.05-2.55)	
≥3	18 (7.4)	33 (6.8)	1.33 (0.72-2.47)	
Effect[3] of prompted[1] event				
Serious illness - spouse	8 (3.3)	16 (3.3)	1.00 (0.42-2.38)	$p = 1.00$
Serious illness - family	20 (8.2)	28 (5.8)	1.47 (0.81-2.66)	$p = 0.213$
Serious illness - close friend	7 (2.9)	3 (0.6)	4.67 (1.21-18.04)	$p = 0.018$
Divorce/separation	6 (2.5)	6 (1.2)	2.00 (0.65-6.20)	$p = 0.235$
Difficulties with family members	55 (22.6)	100 (20.7)	1.11 (0,77-1.60)	$p = 0.572$
Difficulty with neighbours	19 (7.8)	41 (8.5)	0.92 (0.51-1.64)	$p = 0.768$
Serious financial worries	49 (20.2)	84 (17.4)	1.19 (0.81-1.77)	$p = 0.380$
Moving house	4 (1.5)	7 (1.5)	1.14 (0.33-3.90)	$p = 0.832$
Difficulties at work/unemployment	52 (21.4)	103 (21.3)	0.98 (0.65-1.50)	$p = 0.943$
Effect[3] of unprompted[2] events/feelings				
Arguments with spouse/ex-spouse	6 (2.5)	12 (2.5)	1.00 (0.38-2.66)	$p = 1.00$
Problems with accommodation / builders	13 (5.4)	14 (2.9)	1.91 (0.88-4.14)	$p = 0.105$
Court cases/trouble with the law	1 (0.4)	5 (1.0)	0.35 (0.04-3.36)	$p = 0.320$
Organising events (weddings, parties etc)	1 (0.14)	0	-	
Continuing bereavement	10 (4.2)	6 (1.2)	3.33 (1.21-9.17)	$p = 0.017$
Concerns about own health	32 (13.2)	54 (11.2)	1.24 (0.76-2.04)	$p = 0.395$
Feeling isolated from family or friends	8 (3.3)	11 (2.3)	1.49 (0.58-3.81)	$p = 0.413$

[1] Responses to prompted questions [2] Responses to open question [3] Compared to not experiencing the event

Table 6.5.4: Univariable effects of recent illnesses and therapies on the risk of zoster (n=729)

Illness	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
Any major medical or surgical condition in last 6m:	168 (69.1)	279 (57.8)	1.74 (1.23-2.46)	$p = 0.001$
Hospitalisations in last 6m:	9 (3.7)	29 (6.0)	0.61 (0.28-1.30)	$p = 0.183$
Hospitalisations in 2m before rash:	3 (1.2)	11 (2.3)	0.52 (0.14-1.95)	$p = 0.310$
Surgery in the last 6m:				
None	229 (93.8)	455 (93.8)	1.00	
Minor/invasive diagnostic procedures	10 (4.1)	16 (3.3)	1.26 (0.56-2.83)	$p = 0.679$
Major (general anaesthetic)	5 (2.1)	14 (2.9)	0.70 (0.25-2.00)	
Surgical procedure in 2m before rash	6 (2.5)	7 (1.4)	1.80 (0.57-5.69)	$p = 0.318$
Psychiatric illness in last 6m:	7 (2.9)	18 (3.7)	0.78 (0.32-1.86)	$p = 0.566$
Current antidepressent/anxiolytic treatment	19 (7.8)	28 (5.8)	1.40 (0.76-2.58)	$p = 0.287$
Illness or treatment associated with altered micronutrient availability/requirement[1]	13 (5.3)	9 (1.9)	3.34 (1.31-8.74)	$p = 0.009$
Illness or treatment possibly associated with impaired immune functioning[2]	6 (2.5)	9 (1.9)	1.33 (0.47-3.75)	$p = 0.589$
Serious infections in the last 6m	19 (7.8)	17 (3.5)	2.30 (1.18-4.48)	$p = 0.015$
Serious infections in 2m before rash	12 (4.4)	12 (2.2)	2.00 (0.90-4.45)	$p = 0.093$
Past history of cancer	3 (1.2)	11 (2.3)	0.55 (0.15-1.96)	$p = 0.326$

[1] Ulcerative colitis, eating disorders, dysphagia resulting in food regurgitation, iron- or folate-deficient anaemia, pregnancy, antiepileptic medication (phenytoin)

[2] Insulin-dependent diabetes mellitus, Down's syndrome, chronic fatigue syndrome, chronic renal failure, pregnancy, oral steroids

m=months

Table 6.5.5: Multivariable analyses - effects of stressful events or feelings in last 12 months (n=726)

Events/feelings in last 12m	Univariable OR	Adjusted for specific illnesses[1]	Adjusted for any recent illness
All events/feelings combined:			
None	1.00	1.00	1.00
1	1.62 (0.90-2.90)	1.56 (0.86-2.86)	1.56 (0.87-2.79)
2	1.92 (1.09-3.37)	1.83 (1.03-3.28)	1.84 (1.04-3.23)
3	2.19 (1.17-4.08)	2.04 (1.08-3.86)	1.96 (1.05-3.86)
4	2.50 (1.28-4.88)	2.73 (1.37-5.45)	2.47 (1.26-4.87)
≥5	3.20 (1.64-6.24)	2.98 (1.49-5.94)	3.03 (1.55-5.94)
	p=0.012 p(t)=0.0003	p=0.02 p(t)=0.0004	p=0.022 p(t)=0.0005
Individual stress categories			
No. of prompted events:		**Adjusted for other stress variables & illnesses[1]**	
None	1.00	1.00	
1	0.94 (0.59-1.49)	0.86 (0.59-1.49)	
2	1.46 (0.90-2.38)	1.43 (0.90-2.38)	
3	1.31 (0.75-2.28)	1.20 (0.75-2.28)	
4	1.41 (0.71-2.79)	1.31 (0.64-2.66)	
≥5	2.26 (1.12-4.54)	2.32 (1.12-4.82)	
	p=0.124 p(t)=0.014	p=0.099 p(t)=0.022	
No. of unprompted events:			
None	1.00	1.00	
1	2.02 (1.31-3.11)	1.88 (1.21-2.93)	
2	1.65 (0.58-4.65)	1.79 (0.75-4.26)	
	p=0.003 p(t)=0.0009	p=0.011 p(t)=0.005	
Stressful feelings:			
No	1.00	1.00	
Yes	1.56 (1.08-2.26)	1.64 (1.09-2.46)	
	p = 0.021	p = 0.018	
Illnesses			
Any medical/surgical condition in last year:		**Adjusted for stress variables**	**Adjusted for stress variables & illnesses[1]**
No	1.00	1.00	1.00
Yes	1.74 (1.23-2.46)	1.54 (1.07-2.21)	1.57 (1.08-2.29)
	p = 0.001	p = 0.018	p = 0.017
Conditions → altered micronutrient availability:		**Adjusted for stress variables & other illnesses[1]**	
No	1.00	1.00	
Yes	3.34 (1.31-8.74)	2.42 (0.97-6.06)	
	p = 0.009	p = 0.054	
Serious infections in last 6m:			
No	1.00	1.00	
Yes	1.30 (1.18-4.48)	2.06 (1.00-4.23)	
	p = 0.015	p = 0.049	
Hospitalisation in last 6m:			
No	1.00	1.00	
Yes	0.61 (0.28-1.30)	0.38 (0.16-0.87)	
	p = 0.183	p = 0.016	

[1]Conditions associated with altered micronutrient availability/requirement, serious infections & hospitalisations in last 6m

Table 6.5.6: Effect of stressful events/feelings in the last year on the risk of zoster, by age

	Age < 60y (n=390)[1]	Age ≥ 60y (n=336)[1]
Any stressful event/feeling:		
None	1.00	1.00
1	0.40 (0.14-1.08)	2.68 (1.24-5.81)
2	0.47 (0.18-1.21)	3.24 (1.49-7.01)
3	0.34 (0.12-0.94)	6.51 (2.58-16.39)
4	0.72 (0.12-0.94)	3.68 (1.27-10.69)
≥5	0.82 (0.26-1.99)	1.77 (1.12-12.71)
	p=0.038	p=0.0009
No of prompted stressful events:	**Age < 60y (n=390)[2]**	**Age ≥ 60y (n=336)[2]**
None	1.00	1.00
1	0.33 (0.14-0.80)	1.19 (0.66-2.16)
2	0.51 (0.22-1.17)	2.23 (1.09-4.56)
3	0.40 (0.16-1.00)	3.32 (1.31-8.41)
4	0.53 (0.20-1.38)	2.07 (0.49-8.74)
≥5	0.94 (0.47-3.64)	1.44 (0.22-9.35)
	p=0.058	p=0.069

[1] Adjusted for medical conditions [2]Adjusted for number of unprompted events, stressful feelings, medical conditions

Table 6.5.7: Multivariable analysis - effect of stressful events or feelings in the 2 months before rash onset (n=726)

Events/feelings	Univariable OR	Adjusted for other stress variable & medical conditions	
All incident events/feelings:			
None	1.00	1.00	
1	2.36 (1.59-3.48)	2.34 (1.56-3.50)	
≥2	3.50 (1.75-6.98)	3.20 (1.57-6.49)	
	$p<0.0001$ $p(t)<0.0001$	$p<0.0001$ $p(t)<0.0001$	
All prevalent events/feelings:			
None	1.00	1.00	
1	1.44 (0.99-2.10)	1.25 (0.84-1.86)	
2	2.05 (1.30-3.23)	1.80 (1.11-2.88)	
≥3	1.67 (0.98-2.85)	1.49 (0.86-2.60)	
	$p = 0.013$	$p = 0.093$	
Individual stress categories			
≥1 *incident prompted event:*		**Adjusted for other stress variables & medical conditions**	
No	1.00	1.00	
Yes	2.16 (1.46-3.19)	2.17 (1.44-3.27)	
	p=0.0001	p=0.0002	
≥1 *incident unprompted event:*			
No	1.00	1.00	
Yes	4.05 (2.09-7.87)	3.76 (1.90-7.42)	
	$p<0.0001$	p=0.0001	
≥1 *prevalent stressful feeling:*			
No	1.00	1.00	
Yes	1.53 (1.01-2.31)	1.48 (0.95-2.29)	
	p= 0.046	p=0.08	
Illnesses			
Any medical/surgical condition in the last year:		**Adjusted for stress variables**	**+ adjusted for food-related illnesses[1]**
No	1.00	1.00	1.00
Yes	1.74 (1.23-2.46)	1.64 (1.14-2.37)	1.61 (1.11-2.32)
	$p = 0.001$	$p = 0.007$	$p = 0.011$
Conditions → altered micronutrient availability:			
No	1.00	1.00	
Yes	3.34 (1.31-8.74)	2.60 (1.04-6.50)	
	$p = 0.009$	$p = 0.037$	

[1] Conditions associated with altered micronutrient availability/requirement

Table 6.6.1.: Effect of physical trauma (including surgery) on the risk of zoster (n=726)

Physical trauma	CASES n (%)	CONTROLS n (%)	Univariable OR[1] (95% CI)	Adjusted for current illness
Last 6 months				
At any site:				
No	182 (74.9)	370 (76.6)	1.00	1.00
Yes	61 (25.2)	113 (23.4)	1.10 (0.77-1.58) $p = 0.598$	1.03 (0.72-1.49) $p = 0.864$
At same site as rash:				
No	226 (93.0)	478 (99.0)	1.00	1.00
Yes	17 (7.0)	5 (1.0)	10.38 (3.02-35.62) $p < 0.0001$	9.61 (2.79-33.17) $p < 0.0001$
At different site to rash:				
No	198 (81.5)	373 (77.2)	1.00	1.00
Yes	45 (18.5)	110 (22.8)	0.77 (0.52-1.14) $p = 0.182$	0.72 (0.49-1.08) $p = 0.105$
Month before rash onset				
At any site:				
No	217 (89.3)	457 (94.6)	1.00	1.00
Yes	26 (10.7)	26 (5.4)	2.28 (1.25-4.18) $p = 0.007$	2.24 (1.22-4.11) $p = 0.009$
At same site as rash:				
No	233 (95.5)	480 (99.4)	1.00	1.00
Yes	11 (4.5)	3 (0.6)	19.08 (2.44-149.10) p =0.0001	18.15 (2.32-142.31) p =0.0001
At different site to rash:				
No	228 (93.8)	459 (95.0)	1.00	1.00
Yes	15 (6.2)	22 (5.0)	1.28 (0.65-2.55) $p = 0.478$	1.27 (0.64-2.52) $p = 0.499$

Table 6.6.2: ***Effect[1] of physical trauma in the last month on risk of zoster, by age***

Trauma in month before rash	Age < 60y (n=390)	Age ≥ 60y (n=336)
At any site:		
No	1.00	1.00
Yes	1.09 (0.47-2.55) p=0.840	6.22 (2.05-18.86) p=0.001
	(n=381)[2]	**(n=311)[2]**
At different site to rash:		
No	1.00	1.00
Yes	0.78 (0.30-2.03) p=0.601	4.23 (1.11-16.09) p=0.022

[1] Adjusted for current illness

[2] Excluding individuals with trauma to the same site as rash

Table 6.7.1: Effect of selected potential confounders on the risk of zoster

Confounder	CASES n (%)	CONTROLS n (%)	OR (95% CI)	
Smoking status (n=726):				
Non-smoker	90 (37.0)	181 (37.5)	1.00	
Ex-smoker	87 (35.8)	142 (29.4)	1.26 (0.86-1.85)	$p = 0.119$
Current smoker	66 (27.2)	160 (33.1)	0.82 (0.55-1.22)	$p(t) = 0.375$
Cigarettes/day in last yr (n=726):				
None	179 (73.7)	332 (68.7)	1.00	
1-9	23 (9.5)	60 (12.4)	0.71 (0.42-1.20)	$p = 0.460$
10-19	20 (8.2)	50 (10.4)	0.75 (0.43-1.29)	$p(t) = 0.362$
≥20	21 (8.6)	41 (8.5)	0.92 (0.53-1.61)	
Daily alcohol intake in the last yr(g) (n=726):				
None	64 (26.3)	130 (26.9)	1.00	
0.4 - 4.8	34 (14.0)	92 (19.1)	0.76 (0.46-1.24)	$p = 0.300$
4.9 - 12.1	56 (23.1)	85 (17.6)	1.34 (0.85-2.09)	$p(t) = 0.516$
12.2 - 28.6	43 (17.7)	89 (18.4)	0.99 (0.60-1.63)	
28.7 - 173.1	46 (18.9)	87 (18.0)	1.06 (0.62-1.82)	
Housing tenure (n=729):				
Owner occupier	140 (57.4)	266 (54.8)	1.00	
Rents - council	62 (25.4)	142 (29.3)	0.80 (0.53-1.20)	$p = 0.722$
Rents – housing association	17 (7.0)	28 (5.8)	1.15 (0.58-2.25)	
Rents – private	24 (9.8)	45 (9.3)	1.01 (0.58-1.75)	
Other	1 (0.4)	4 (0.8)	0.50 (0.06-4.47)	
Cars in the household (n=729):				
None	90 (36.9)	196 (40.4)	1.00	$p = 0.543$
One	116 (47.5)	217 (44.7)	1.23 (0.84-1.80)	$p(t) = 0.354$
Two or more	38 (15.6)	72 (14.9)	1.24 (0.73-2.11)	

$p(t) = p$ value for trend

Table 6.8.1: Independent effect of variables on risk of zoster (combined model) (n = 628)

Variable		Adjusted OR (revised sub-model)	Adjusted OR (combined model)[1]
Contacts with specific children not resident in household (last 10y):			
	None	1.00	1.00
	1-107	1.09 (0.56-2.13)	1.06 (0.48-2.34)
	108-420	0.88 (0.45-1.73)	0.73 (0.32-1.65)
	421-1334	0.92 (0.46-1.84)	0.91 (0.39-2.10)
	1335-3457	0.69 (0.33-1.45)	0.61 (0.25-1.48)
	3458-32631	0.38 (0.16-0.91) $p = 0.08^h, 0.01^t$	0.28 (0.10-0.76) $p = 0.03^h; 0.005^t$
Contacts with groups of children (last 10y):			
	None	1.00	1.00
	1-550	0.70 (0.38-1.30)	0.84 (0.41-1.72)
	551-3652	0.48 (0.23-0.98)	0.42 (0.18-0.97)
	3653-7492	0.24 (0.09-0.66) $p < 0.001^{h+t}$	0.25 (0.08-0.82) $p = 0.032^h; 0.004^t$
Occupational contact - ill children (last 10y)			
	None	1.00	1.00
	Up to 5 years	0.26 (0.05-1.24)	0.12 (0.02-0.77)
	More than 5 years	0.26 (0.03-2.43) $p = 0.012^h$	0.16 (0.13-1.90) $p = 0.014^h; 0.006^t$
Contacts with varicella (last 10y):			
	None	1.00	1.00
	1	0.93 (0.54-1.60)	0.91 (0.49-1.69)
	2	0.81 (0.43-1.56)	0.75 (0.35-1.65)
	3 - 4	0.22 (0.07-0.66)	0.17 (0.06-0.64)
	5+	0.30 (0.10-0.90) $p=0.014^h, 0.003^t$	0.24 (0.07-0.86) $p = 0.017^h, 0.003^t$
Ethnicity:	White	1.00	1.00
	Afrocaribbean	0.48 (0.23-1.04)	0.92 (0.33-2.59)
	Asian	2.27 (0.70-7.38)	2.41 (0.50-11.69)
	Other	0.95 (0.31-2.93) $p=0.10^h$	1.64 (0.37-7.23) $p=0.64^h$
Fresh fruit intake(last year):			
	> 3 portions per day	1.00	1.00
	2 - 3 portions per day	1.91 (1.14-3.21)	2.05 (1.06-3.97)
	5 - 7 portions per week	2.18 (1.29-3.68)	2.61 (1.32-5.14)
	1 - 4 portions per week	2.60 (1.38-4.90)	3.43 (1.51-7.76)
	< 1 portion per week	3.80 (1.64-8.79) $p = 0.005^h, 0.0003^t$	6.66 (2.14-20.67) $p = 0.003^h, 0.0001^t$
Childhood weekly summer non-holiday UVR:			
	3.0-16.2	1.00	1.00
	16.2-20.5	2.68 (1.45-4.96)	3.79 (1.78-8.07)
	20.5-23.9	2.73 (1.49-5.00)	4.51 (2.05-9.93)
	23.9-27.4	2.43 (1.30-4.54)	4.96 (2.25-10.95)
	27.4-84.2	1.82 (0.96-3.48) $p = 0.006^h, 0.0009^q$	2.42 (1.09-5.38) $p = 0.0002^h, <0.0001^q$
Years since varicella:			
	>50 years	1.00	1.00
	31 - 50 years	1.44 (0.68-3.10)	1.32 (0.51-3.39)
	11 - 30 years	0.72 (0.20-1.75)	0.45 (0.14-1.45)
	≤ 10 years	0.09 (0.01-0.97)	0.05 (0.00-0.92)
	No history of varicella	1.45 (0.88-2.37) $p = 0.022^h$	1.33 (0.70-2.53) $p = 0.053^h$
Prompted incident stress event in last 2m		2.12 (1.36-3.32) $p=0.0008$	2.31 (1.32-4.04) $p=0.003$
Unprompted incident stress event in last 2m		3.60 (1.70-7.61) $p=0.0005$	5.65 (2.14-14.94) $p=0.0002$
Prevalent stressful feeling in last 2m		1.77 (1.10-2.84) $p=0.018$	2.00 (1.10-3.63) $p=0.021$
Current illness (vs not)		1.57 (1.08-2.30) $p=0.018$	1.71 (1.08-2.73) $p=0.02$

[1]Adjusted for other variables in the model+smoking [h] *p* value (heterogeneity) [t] *p* value (trend) [q] *p* value (quadratic)

Table 6.8.2: Combined model – independent effects of alternative variables (n=628)

Variable	Adjusted OR (revised sub-model)	Adjusted OR (combined model[1])
Child/varicella contacts in last year		
Contacts with specific children not resident in household:		
None	1.00	1.00
1-11	0.84 (0.45-1.56)	0.94 (0.43-2.06)
12-52	1.37 (0.79-2.38)	1.25 (0.62-2.52)
53-155	0.88 (0.48-1.59)	0.79 (0.38-1.64)
156-381	0.65 (0.33-1.28)	0.55 (0.24-1.26)
382-3650	0.50 (0.23-1.09)	0.40 (0.15-1.04)
	p=0.09[h];0.063[t]	p=0.123[h];0.022[t]
Contacts with groups of children:		
None	1.00	1.00
1-312	0.83 (0.45-1.51)	0.73 (0.36-1.48)
313-993	0.18 (0.07-0.42)	0.26 (0.08-0.83)
	p=0.023[h];0.013[t]	p=0.04[h];0.016[t]
Occupational contact with ill children:		
No	1.00	1.00
Yes	0.42 (0.07-2.44) p=0.304	0.21 (0.02-1.86) p=0.128
Contacts with varicella:		
None	1.00	1.00
1	1.92 (0.96-3.84)	3.61 (1.50-8.68)
2+	0.38 (0.13-1.09) p=0.02[h]	0.41 (0.13-1.28) p=0.005[h]
Fresh/frozen fruit & vegetables in last yr		
≥ 8 portions per day	1.00	1.00
6 - 7 portions per day	1.66 (0.92-2.99)	1.74 (0.84-3.61)
4 - 5 portions per day	2.37 (1.36-4.13)	2.53 (1.27-5.05)
2 - 3 portions per day	2.84 (1.55-5.21)	3.08 (1.42-6.65)
≤ 1 portion per day	3.28 (1.43-7.53)	3.75 (1.23-11.43)
	p=0.003[h];0.0001[t]	p=0.024[h];0.001[t]
Total UVR exposure (month before rash):		
0 - 3.06	1.00	1.00
3.06 - 11.40	2.52 (1.32-4.80)	2.55 (1.20-5.42)
11.40 - 33.33	1.94 (0.88-4.24)	1.79 (0.69-4.63)
33.33 - 64.02	2.59 (1.12-5.99)	3.66 (1.33-10.07)
64.02 - 293.46	2.92 (1.24-6.90) p=0.039[h]	3.59 (1.30-9.90) p=0.022[h]
Stress/Illness in the last year:		
No. of prompted stress events:		
None	1.00	1.00
1	0.96 (0.58-1.57)	0.83 (0.45-1.54)
2	1.62 (0.96-2.76)	1.80 (0.90-3.62)
3	1.40 (0.77-2.54)	1.63 (0.78-3.44)
4	1.68 (0.82-3.42)	1.61 (0.61-4.24)
≥5	2.41 (1.10-5.28)	6.96 (2.35-20.56)
	p=0.101[h];0.011[t]	p=0.003[h];0.0008[t]
No. of unprompted stress events:		
None	1.00	1.00
1	1.77 (1.13-2.77)	2.08 (1.14-3.82)
2	2.10 (0.88-5.03)	1.73 (0.55-5.41)
	p=0.016[h];0.005[t]	p=0.042[h];0.024[t]
Stressful feelings:	1.80 (1.20-2.69) p=0.0005	1.76 (1.02-3.06) p=0.022
Illnesses → altered micronutrient availability	2.36 (0.83-6.70) p=0.098	3.67 (1.05-12.82) p=0.039

[1]Adjusted for variables from other sub-models, as listed in Table 6.8.1

Table 6.8.3: Independent effects of selected variables, by age – controlling for confounders from other sub-models

Exposure	Age < 60 years	Age ≥ 60 years
Micronutrient score[1]*:*		
27 – 35		1.00
23 – 26	(N/S)	3.67 (0.81-16.55)
20 – 22		9.65 (2.51-37.09)
16 - 19		7.44 (1.97-28.03)
7– 15		20.31 (4.64-88.84)
		$p<0.0001^{h+t}$
Summer holiday UVR exposure (childhood)[2]		
No holidays	2.83 (0.83-9.62)	
2.80 - 39.2	1.00	(N/S)
39.3 – 61.1	1.92 (0.49-7.45)	
61.2 - 84.6	3.87 (1.08-13.90)	
84.7 – 160.2	4.11 (1.14-14.87)	
160.3 – 1037.2	3.06 (0.79-11.86)	
	$p= 0.203^{h}$	
Prompted stress events (last yr)[3,4]*:*		
None	1.00	1.00
1	0.27 (0.08-0.83)	1.18 (0.56-2.49)
2	0.46 (0.16-1.37)	2.74 (1.08-6.96)
3	0.28 (0.09-0.95)	6.00 (1.86-19.40)
≥4	1.10 (0.36-3.38)	3.39 (0.71-16.16)
	$p= 0.003^{h}$	$p=0.006^{h}$

[1]Adjusted for contacts in last 10y with groups of children + specific children not living in the household, total weekly non-holiday UVR exposure in warmer months of childhood, current illness, incident prompted + unprompted stress events in the 2 months before rash (n=278)

[2]Adjusted for weekly non-holiday UVR exposure in warmer months of childhood, ethnicity, years since varicella and contacts with varicella cases in last 10y (n=344).

[3]Individuals aged <60yrs: adjusted for unprompted stress events & feelings in last 12m, current illness, contacts with varicella cases and no of years since varicella (n=350)

[4]Individuals aged ≥ 60yrs: adjusted for unprompted stress events & feelings in last 12m, current illness, fruit intake, contacts with children not living in the household (n=278)

[h] *p* value (heterogeneity) [t] *p* value (trend) [q] *p* value (quadratic)
N/S: variable not significantly associated with risk of zoster in this age group

9. REFERENCES

1 Miller E, Marshall R, Vurdien J. Epidemiology, outcome and control of varicella-zoster infection. *Rev Med Microbiol* 1993; **4**:222-230.

2 Brisson M, Edmunds WJ, Law B *et al.* Epidemiology of varicella zoster virus infection in Canada and the United Kingdom. *Epidemiol Infect* 2001; **127**:305-314.

3 Kost RG, Straus SE. Postherpetic neuralgia - pathogenesis, treatment, and prevention. *N Engl J Med* 1996; **335**:32-42.

4 Ragozzino MW, Melton III LJ, Kurland LT, Chu CP, Perry HO. Population-based study of herpes zoster and its sequelae. *Medicine* 1982; **61**:310-316.

5 Edmunds WJ, Brisson M, Rose JD. The epidemiology of herpes zoster and the potential cost-effectiveness of vaccination in England and Wales. *Vaccine* 2001; **19**:3076-3090.

6 Jackson JL, Gibbons R, Meyer G, Inouye L. The effect of treating herpes zoster with oral acyclovir in preventing postherpetic neuralgia: a meta-analysis. *Arch Intern Med* 1997; **157**:909-912.

7 Alper BS, Lewis PR. Does treatment of acute herpes zoster prevent or shorten postherpetic neuralgia? *J Gen Virol* 2001; **49**:255-264.

8 Nikkels AF, Pierard GE. Oral antivirals revisited in the treatment of herpes zoster: what do they accomplish? *Am J Clin Dermatol* 2002; **3**:591-598.

9 Burke IL, Steele RW, Beard OW *et al.* Immune responses to varicella-zoster in the aged. *Arch Intern Med* 1982; **142**:291-293.

10 Berger R, Florent G, Just M. Decrease of the lymphoproliferative response to varicella-zoster virus antigen in the aged. *Infect Immun* 1981; **32**:24-27.

11 Saibara T, Maeda T, Onishi S, Yamamoto Y. Depressed immune functions in the early phase of varicella-zoster virus reactivation. *J Med Virol* 1993; **39**:242-245.

12 Sørensen OS, Haahr S, Moller-Larsen A, Wildenhoff K. Cell-mediated and humoral immunity to herpesviruses during and after herpes zoster infections. *Infect Immun* 1980; **29**:369-375.

13 Morens DM, Bregman DJ, West CM *et al.* An outbreak of varicella-zoster virus infection among cancer patients. *Ann Intern Med* 1980; **93**:414-419.

14 Alliegro MB, Dorrucci M, Pezzotti P *et al.* Herpes zoster and progression to AIDS in a cohort of individuals who seroconverted to human immunodeficiency virus. *Clin Infect Dis* 1996; **23**:990-995.

15 Nagasawa K, Yamauchi Y, Tada Y *et al.* High incidence of herpes zoster in patients with systemic lupus erythematosus: an immunological analysis. *Ann Rheum Dis* 1990; **49**:630-633.

16 Donahue JG, Choo PW, Manson JE, Platt R. The incidence of herpes zoster. *Arch Intern Med* 1995; **155**:1605-1609.

17 Guess HA, Broughton DD, Melton III LJ, Kurland LT. Epidemiology of herpes zoster in children and adolescents: a population-based study. *Pediatrics* 1985; **76**:512-517.

18 McGregor RM. Herpes zoster, chicken-pox, and cancer in general practice. *BMJ* 1957; **i**:84-87.

19 Ross CA, Brown WK, Clarke A *et al.* Herpes zoster in general practice. *J R Coll Gen Pract* 1975; **25**:29-32.

20 Seiler HE. A study of herpes zoster particularly its relationship to chickenpox. *J Hyg* 1949; **47**:253-262.

21 Wilson JB. Thirty one years of herpes zoster in a rural practice. *BMJ* 1986; **293**:1349-1351.

22 Richards P. Shingles in one family practice. *Arch Fam Med* 1996; **5**:42-46.

23 McCormick A, Fleming D, Charlton J. Morbidity Statistics from General Practice: Fourth National Study 1991-92. A study carried out by the Royal College of General Practitioners, the Office of Population Censuses and Surveys, and the Department of Health. London: HMSO, 1995.

24 Gershon AA. Live-attenuated varicella vaccine. *Infect Dis Clin North Am* 2001; **15**:65-81.

25 Garnett GP, Grenfell BT. The epidemiology of varicella-zoster virus infections: a mathematical model. *Epidemiol Infect* 1992; **108**:495-511.

26 Brisson M, Edmunds WJ, Gay NJ, Law B, Dean JH. Modelling the impact of immunization on the epidemiology of varicella zoster virus. *Epidemiol Infect* 2000; **125**:651-669.

27 Davison AJ, Scott JE. The complete DNA sequence of varicella-zoster virus. *J Gen Virol* 1986; **67**:1759-1816.

28 Gilden DH, Vafai A, Shtram Y *et al.* Varicella-zoster virus DNA in human sensory ganglia. *Nature* 1983; **306**:478-480.

29 Furuta Y, Takasu T, Fukuda S *et al.* Detection of varicella-zoster virus DNA in human geniculate ganglia by polymerase chain reaction. *J Infect Dis* 1992; **166**:1157-1159.

30 Kennedy PGE, Grinfeld E, Gow JW. Latent varicella-zoster virus is located predominantly in neurons in human trigeminal ganglia. *Proc Natl Acad Sci USA* 1998; **95**:4658-4662.

31 Miller AE. Selective decline in cellular immune response to varicella zoster in the elderly. *Neurology* 1980; **30**:582-587.

32 Arvin AM, Pollard RB, Rasmussen LE, Merigan TC. Cellular and humoral immunity in the pathogenesis of recurrent herpes viral infections in patients with lymphoma. *J Clin Invest* 1980; **65**:869-878.

33 Lungu O, Annunziato PW. Varicella-zoster virus: latency and reactivation. *Contrib Microbiol* 1999; **3**:61-75.

34 Hope-Simpson RE. The nature of herpes zoster: a long-term study and a new hypothesis. *Proc Royal Soc Med* 1965; **58**:9-20.

35 Gelb LD. Varicella-zoster virus: clinical aspects. In: Roizman B, Whitley RJ, Lopez C (Editors). The Human Herpesviruses. New York: Raven Press, 1993.

36 Rubben A, Baron JM, Grussendorf-Conen EI. Routine detection of herpes simplex virus and varicella zoster virus by polymerase chain reaction reveals that initial herpes zoster is frequently misdiagnosed as herpes simplex. *Br J Dermatol* 1997; **137**:259-261.

37 Gnann JW. Varicella-zoster virus: atypical presentations and unusual complications. *J Infect Dis* 2002; **186** (Suppl 1):S91-S98.

38 Yi JY, Kim TY, Shim JH, Cho BK, Kim CW. Histopathological findings, viral DNA distribution and lymphocytic immunophenotypes in vesicular and papular types of herpes zoster. *Acta Derm Venereol* 1997; **77**:194-197.

39 Glesby MJ, Moore RD, Chaisson RE. Clinical spectrum of herpes zoster in adults infected with human immunodeficiency virus. *Clin Infect Dis* 1995; **21**:370-375.

40 Watson CPN, Deck JH, Morshead C, Van der Kooy D, Evans RJ. Post-herpetic neuralgia: further post-mortem studies of cases with and without pain. *Pain* 1991; **44**:105-117.

41 Chern KC, Margolis TP. Varicella zoster virus ocular disease. *Int Ophthalmol Clin* 1998; **38** :149-160.

42 Gnann JW, Whitley RJ. Natural history and treatment of varicella-zoster in high-risk populations. *J Hosp Infect* 1991; **18** (Suppl A):317-329.

43 Whitley RJ, Roizman B. Herpex simplex virus infections. *Lancet* 2001; **357**:1513-1518.

44 Kalman CM, Laskin OL. Herpes zoster and zosteriform herpes simplex virus infections in immunocompetent adults. *Am J Med* 1986; **81**:775-778.

45 Sandor EV. Herpes zoster and zosteriform herpes simplex virus infections in immunocompetent adults. *Am J Med* 1987; **82**:861-862.

46 Zajdowicz TR, Chang MJ. Herpes zoster and zosteriform herpes simplex virus infections in immunocompetent adults. *Am J Med* 1987; **82**:862.

47 Solomon AR, Rasmussen JE, Weiss JS. A comparison of the Tzanck smear and viral isolation in varicella and herpes zoster. *Arch Dermatol* 1986; **122**:282-285.

48 Nahass GT, Goldstein BA, Zhu WY *et al.* Comparison of Tzanck smear, viral culture and DNA diagnostic methods in detection of herpes simplex and varicella-zoster infection. *JAMA* 1992; **18**:2541-2544.

49 Nahass GT, Mandel MJ, Cook S, Fan W, Leonardi CL. Detection of herpes simplex and varicella-zoster infection from cutaneous lesions in different clinical stages with the polymerase chain reaction. *J Am Acad Dermatol* 1995; **32**:730-733.

50 Eis-Hubinger-A.M., Kaiser R, Kleim JP *et al.* [Detection of varicella zoster virus infections using polymerase chain reaction]. *Hautarzt* 1992; **43**:767-771.

51 The Research Committee of the Council of the College of General Practitioners. General Register Office. Studies on Medical and Population Subjects No 14. Morbidity Statistics from General Practice. London: HMSO, 1962.

52 Royal College of General Practitioners, Office of Population Censuses and Surveys, Department of Health and Social Security. Morbidity Statistics from General Practice 1971-72. Second National Study. Studies on Medical and Population Subjects No. 36. London: HMSO, 1979.

53 Royal College of General Practitioners, Office of Population Censuses and Surveys, Department of Health and Social Security. Morbidity Statistics from General Practice 1981-82. Third National Study. Series MB5 no.1. London: HMSO, 1986.

54 Fleming DM. Weekly returns service of the Royal College of General Practitioners. *Communicable Disease and Public Health* 1999; **2**:96-100.

55 Christensen P, Nørrelund N. [Herpes zoster in general practice]. *Ugeskr Læger* 1985; **147**:3401-3403.

56 Trollor J. Herpes zoster in general practice. *Aust Fam Physician* 1987; **16**:1133-1140.

57 Chant KG, Sullivan EA, Burgess MA *et al.* Varicella-zoster virus infection in Australia. *Aust N Z J Public Health* 1998; **22**:413-418.

58 Meister W, Neiss A, Gross G *et al.* Demography, symptomatology, and course of disease in ambulatory zoster patients. *Intervirology* 1998; **41**:272-277.

59 Schoub BD, Sacho H. Herpes zoster - results from a questionnaire-based surveillance of patients and their general practitioners. *SAMJ* 1996; **86**:1562-1563.

60 Kurland LT. Descriptive epidemiology of selected neurological and myopathic disorders with particular reference to a survey in Rochester, Minnesota. *J Chron Dis* 1958; **8**:378-418.

61 Schmader K, George LK, Burchett BM, Pieper CF, Hamilton JD. Racial differences in the occurrence of herpes zoster. *J Infect Dis* 1995; **171**:701-704.

62 Fleming DM, Norbury CA, Crombie DL. Annual and Seasonal Variation in the Incidence of Common Diseases. [53]. Birmingham: RCGP, 1991.

63 Fleming DM, Ross AM. Weekly Returns Service: Report for 2001. Birmingham: RCGP, 2002.

64 Paul E, Thiel T. [Epidemiology of varicella-zoster infections. Results of a prospective study in Ansbach, Germany.] *Hautarzt* 1996; **47**:604-609.

65 Chidiac C, Bruxelle J, Daures J-P *et al.* Characteristics of patients with herpes zoster on presentation to practitioners in France. *Clin Infect Dis* 2001; **33**:62-69.

66 Czernichow S, Dupuy A, Flahault A, Chosidow O. [Herpes zoster: incidence study among 'Sentinel' general practitioners]. *Ann Dermatol Venereol* 2001; **128**:497-501.

67 Di Luzio Paparatti U, Arpinelli F, Visona G. Herpes zoster and its complications in Italy: an observational survey. *J Infection* 1999; **38**:116-119.

68 Helgason S, Sigurdsson JA, Gudmundsson S. The clinical course of herpes zoster: a prospective study in primary care. *Eur J Gen Pract* 1996; **2**:12-16.

69 Petursson G, Helgason S, Gudmundsson S, Sigurdsson JA. Herpes zoster in children and adolescents. *Pediatr Infect Dis J* 1998; **17**:905-908.

70 Opstelten W, Mauritz JW, de Wit NJ *et al.* Herpes zoster and postherpetic neuralgia: incidence and risk indicators using a general practice research database. *Fam Pract* 2002; **19**:471-475.

71 Hope-Simpson RE. Epidemiology of shingles. *J Royal Soc Med* 1991; **84**:184.

72 Glynn C, Crockford G, Gavaghan D *et al.* Epidemiology of shingles. *J Royal Soc Med* 1990; **83**:617-619.

73 Hellgren L, Hersle K. A statistical and clinical study of herpes zoster. *Geront Clin* 1966; **8**:70-76.

74 Simons RDG. Some climatological and other particulars on herpes zoster from the Northern and Southern hemisphere. *Dermatologica* 1951; **103**:109-116.

75 Gallerani M, Manfredini R. Seasonal variation in herpes zoster infection. *Br J Dermatol* 2000; **142**:560-591.

76 Termorshuizen F, Garssen J, Norval M *et al.* A review of studies on the effects of ultraviolet irradiation on the resistance to infections: evidence from rodent infection models and verification by experimental and observational human studies. *Int Immunopharmacol* 2002; **2**:263-275.

77 Fleming DM. Weekly Returns Service: Report for 1998. Birmingham: RCGP, 1999.

78 Logan WPD, Cushion AA. General Register Office. Studies on Medical and Population Subjects No 14. Morbidity Statistics from General Practice. London: HMSO, 1958.

79 Hayward AR, Herberger M. Lymphocyte responses to varicella-zoster virus in the elderly. *J Clin Immunol* 1987; **7**:174-178.

80 Schmader K, George LK, Burchett BM, Hamilton JD, Pieper CF. Race and stress in the incidence of herpes zoster in older adults. *JAGS* 1998; **46**:973-977.

81 Dworkin RH. Racial differences in herpes zoster and age at onset of varicella. *J Infect Dis* 1996; **174**:239-240.

82 Fairley CK, Miller E. Varicella-zoster virus epidemiology - a changing scene? *J Infect Dis* 1996; **174** (Suppl. 3):S314-319.

83 Wutzler P, Farber I, Wagenpfeil S, Bisanz H, Tischer A. Seroprevalence of varicella-zoster virus in the German population. *Vaccine* 2001; **20**:121-124.

84 Salleras L, Dominguez A, Vidal J *et al.* Seroepidemiology of varicella-zoster virus infection in Catalonia (Spain). Rationale for universal vaccination programmes. *Vaccine* 2000; **19**:183-188.

85 Centers for Disease Control and Prevention. Prevention of varicella - recommendations of the Advisory Committee on Immunization Practices. ACIP). *MMWR* 1996; **45**:1-35.

86 Garnett GP, Cox MJ, Bundy DA, Didier JM, St Catharine J. The age of infection with varicella-zoster virus in St Lucia, West Indies. *Epidemiol Infect* 1993; **110**:361-372.

87 Longfield JN, Winn RE, Gibson RL, Juchau SV, Hoffman PV. Varicella outbreaks in Army recruits from Puerto Rico. Varicella susceptibility in a population from the tropics. *Arch Intern Med* 1990; **150**:970-973.

88 Venkitaramen AR, John TJ. The epidemiology of varicella in staff and students of a hospital in the tropics. *Int J Epidemiol* 1984; **13**:502-505.

89 Maretic Z, Cooray MPM. Comparisons between chickenpox in a tropical and a European Country. *J Trop Med Hyg* 1963; **66**:311-315.

90 Migasena S, Simasathien S, Desakorn V *et al.* Seroprevalence of varicella-zoster virus antibody in Thailand. *Int J Infect Dis* 1997; **2**:26-30.

91 Ooi PL, Goh KT, Doraisingham S, Ling AE. Prevalence of varicella-zoster virus infection in Singapore. *SE Asian J Trop Med* 1992; **23**:22-25.

92 Lolekha S, Tanthiphabha W, Sornchai P *et al.* Effect of climatic factors and population density on varicella zoster virus epidemiology within a tropical country. *Am J Trop Med Hyg* 2001; **64**:131-136.

93 Muñoz MT, Torres J, Damasio-Santana L *et al.* Susceptibility to varicella-zoster infection in individuals 1 to 29 years of age in Mexico. *Arch Med Res* 1999; **30**:60-63.

94 O'Grady K-A, Merianos A, Patel M, Gilbert L. High seroprevalence of antibodies to varicella zoster virus in adult women in a tropical climate. *TMIH* 2000; **5**:732-736.

95 Yu AL, Costa JM, Amaku M *et al.* Three year seroepidemiological study of varicella-zoster virus in Sao Paulo, Brazil. *Rev Inst Med Trop Sao Paulo* 2000; **42**:125-128.

96 Bartoloni A, Bartalesi F, Roselli M *et al.* Seroprevalence of varicella-zoster and rubella antibodies among rural populations of the Chaco Region, South-Eastern Bolivia. *TMIH* 2002; **7**:512-517.

97 Mandal BK, Mukherjee PP, Murphy C, Mukherjee R, Naik T. Adult susceptibility to varicella in the tropics is a rural phenomenon due to the lack of previous exposure. *J Infect Dis* 1998; **178** (Suppl 1):S52-54.

98 Taylor-Wiedman J, Yamashita K, Miyamura K, Yamazaki S. Varicella-zoster virus prevalence in Japan: no significant change in a decade. *Japan J Med Sci Biol* 1989; **42**:1-11.

99 Edson CM, Hosler BA, Respress RA. Cross reactivity between herpes simplex virus glycoprotein B and a 63000 Dalton varicella zoster virus envelope glycoprotein. *J Virol* 1985; **56**:333-336.

100 Sinha DP. Chickenpox - a disease predominantly affecting adults in rural West Bengal, India. *Int J Epidemiol* 1976; **5**:367-374.

101 Baba K, Yabuuchi H, Takahashi M, Ogra PL. Increased incidence of herpes zoster in normal children infected with varicella zoster virus during infancy: community-based follow-up study. *J Pediatr* 1986; **108**:372-377.

102 Enders G, Miller E, Cradock-Watson J, Bolley I, Ridehalgh M. Consequences of varicella and herpes zoster in pregnancy: prospective study of 1739 cases. *Lancet* 1994; **343**:1548-1551.

103 Arvin AM, Koropchak CM, Wittek AE. Immunological evidence of re-infection with varicella zoster virus. *J Infect Dis* 1983; **148**:200-205.

104 Ikada J, Kagami K, Yamamoto Y. Varicella may protect against zoster. *Lancet* 1973; **2**:1276.

105 Garnett GP, Grenfell BT. The epidemiology of varicella-zoster virus infections: the influence of varicella on the prevalence of herpes zoster. *Epidemiol Infect* 1992; **108**:513-528.

106 Solomon BA, Kaporis AG, Glass AT, Simon SI, Baldwin HE. Lasting immunity to varicella in doctors study (L.I.V.I.D. study). *J Am Acad Dermatol* 1998; **38**:763-765.

107 Terada K, Hiraga Y, Kawano S, Kataoka N. [Incidence of herpes zoster in pediatricians and history of reexposure to varicella-zoster virus in patients with herpes zoster]. *Kansen Zasshi* 1995; **69**:908-912.

108 Gershon AA, LaRussa P, Steinberg S *et al.* The protective effect of immunologic boosting against zoster: an analysis in leukemic children who were vaccinated against chickenpox. *J Infect Dis* 1996; **173**:450-453.

109 Schimpff S, Serpick A, Stoler B *et al.* Varicella-zoster infection in patients with cancer. *Ann Intern Med* 1972; **76**:241-254.

110 Sokal JE, Firat D. Varicella-zoster infection in Hodgkin's disease. *Am J Med* 1965; **39**:452.

111 Antonelli MAS, Moreland LW, Brick JE. Herpes zoster in patients with rheumatoid arthritis treated with weekly low-dose methotrexate. *Am J Med* 1991; **90**:295-298.

112 Melbye M, Grossman RJ, Goedert JJ, Eyster ME, Biggar RJ. Risk of AIDS after herpes zoster. *Lancet* 1987; **1**:728-730.

113 Samba-Lefevre MC, Kouka F, Yala F *et al.* Herpes zoster and HIV infection in Congo (in French). *Bull Liais Document* 1989; **4**:68.

114 Melbye M, Njelesani EK, Bayley A *et al.* Evidence for heterosexual transmission and clinical manifestations of human immunodeficiency virus infection and related conditions in Lusaka, Zambia. *Lancet* 1986; **2**:1113-1115.

115 Buchbinder SP, Katz MH, Hessol NA *et al.* Herpes zoster and human immunodeficiency virus infection. *J Infect Dis* 1992; **166**:1153-1156.

116 Engels EA, Rosenberg PS, Biggar RJ. Zoster incidence in human immunodeficiency virus-infected hemophiliacs and homosexual men, 1984-1997. *J Infect Dis* 1999; **180**:1784-1789.

117 Morgan D, Mahe C, Malamba S *et al.* Herpes zoster and HIV-1 infection in a rural Ugandan cohort. *AIDS* 2001; **15**:223-229.

118 Luby JP, Ramirez-Ronda C, Rinner S, Hull A, Vergne-Marini P. A longitudinal study of varicella-zoster virus infections in renal transnplant recipients. *J Infect Dis* 1977; **135**:659-663.

119 Rifkind D. The activation of varicella-zoster virus infections by immunosuppressive therapy. *J Lab Clin Med* 1966; **68**:463.

120 Colebunders R, Mann JM, Francis H *et al.* Herpes zoster in African patients: a clinical predictor of human immunodeficiency virus. *J Infect Dis* 1988; **157**:314-318.

121 Van der Perre P, Bakkers E, Batungwanayo J *et al.* Herpes zoster in African patients: an early manifestation of HIV infection. *Scand J Infect Dis* 1989; **20**:277-282.

122 Dehne KL, Dhlakama DG, Richter C *et al.* Herpes zoster as an indicator of HIV infection in Africa. *Trop Doct* 1992; **22**:68-70.

123 Tyndall MW, Basio J, Agoki E *et al.* Herpes zoster as the initial presentation of human immunodeficiency virus type 1 infection in Kenya. *Clin Infect Dis* 1995; **21**:1035-1037.

124 Bayu S, Alemahehu W. Clinical profile of herpes zoster ophthalmicus in Ethiopians. *Clin Infect Dis* 1997; **24**:1256-1260.

125 Ragozzino MW, Melton III LJ, Kurland LT, Chu PH, Perry HO. Risk of cancer after herpes zoster: a population-based study. *N Engl J Med* 1982; **307**:393-397.

126 Stulnig T, Maczek C, Bock G, Majdic O, Wick G. Reference intervals for human peripheral blood lymphocyte subpopulations from 'healthy' young and aged subjects. *Int Arch Allergy Immunol* 1995; **108**:205-210.

127 Gillis S, Durante M, Weksler ME. Immunological studies of aging: decreased production of and response to T cell growth factor by lymphocytes from aged humans. *J Clin Invest* 1981; **67**:937-942.

128 Kudlacek S, Jahandideh-Kazempour S, Graninger W, Willvonseder R, Pietschmann P. Differential expression of various T cell surface markers in young and elderly subjects. *Immunobiol* 1995; **192**:198-204.

129 Rae IM, Alexander HD, Crockard AD, Morris TC. CD4 lymphopenia in very elderly people. *Lancet* 1996; **347**:328-329.

130 Goodwin JS, Searles RP, Tung SK. Immunological responses of a healthy elderly population. *Clin Exp Immunol* 1982; **48**:403-410.

131 Bradley J. Diet, age, and the immune system. *Nutr Rev* 1996; **54**:S43-50.

132 Castle SC. Clinical relevance of age-related immune dysfunction. *Clin Infect Dis* 2000; **31**:578-585.

133 Chandra RK. Nutritional regulation of immunity and risk of infection in old age. *Immunology* 1989; **67**:141-147.

134 Barcellini W, Borghi MO, Sguotti C. Heterogeneity of immune responsiveness in healthy elderly subjects. *Clin Immunol Immunopathol* 1988; **47**:142-151.

135 Kusnecov AW, Rabin BS. Stressor-induced alterations of immune function: mechanisms and issues. *Int Arch Allergy Immunol* 1994; **105**:107-121.

136 Bartrop RW, Luckhurst E, Lazarus L, Kiloh LG, Penny R. Depressed lymphocyte function after bereavement. *Lancet* 1977; **i**:834-836.

137 Schleifer SJ, Keller SE, Camerino M, Thornton JC, Stein M. Suppression of lymphocyte stimulation following bereavement. *JAMA* 1983; **250**:374-377.

138 Maes M, Van Bockstaele DR, Gastel A *et al.* The effects of psychological stress on leukocyte subset distribution in humans: evidence of immune activation. *Neuropsychobiol* 1999; **39**:1-9.

139 Dorian B, Garfinkel P, Brown G *et al.* Aberrations in lymphocyte subpopulations and function during psychological stress. *Clin Exp Immunol* 1982; **50**:132-138.

140 Bauer ME, Vedhara K, Perks P *et al.* Chronic stress in caregivers of dementia patients is associated with reduced lymphocyte sensitivity to glucocorticoids. *J Neuroimmunol* 2000; **103**:84-92.

141 Kiecolt-Glaser JK, Glaser R, Gravenstein S, Malarkey WB. Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proc Natl Acad Sci USA* 1996; **93**:3043-3047.

142 Irwin M, Costlow C, Williams H *et al.* Cellular immunity to varicella-zoster virus in patients with major depression. *J Infect Dis* 1998; **178** (Suppl 1):S104-108.

143 Dhabhar FS, McEwen BS. Enhancing versus suppressive effects of stress hormones on skin immune function. *Proc Natl Acad Sci USA* 1999; **96**:1059-1064.

144 Kang DH, Coe CL, McCarthy DO, Ershler WB. Immune responses to final exams in healthy and asthmatic adolescents. *Nurs Res* 1997; **46**:12-19.

145 Cohen F, Kearney KA, Zegans LS *et al.* Differential immune system changes with acute and persistent stress for optimists vs pessimists. *Brain Behav Immun* 1999; **13**:155-174.

146 Olff M. Stress, depression and immunity: the role of defense and coping styles. *Psychiatry Res* 1999; **85**:7-15.

147 Cohen S, Tyrrell DAJ, Smith AP. Psychological stress and susceptibility to the common cold. *N Engl J Med* 1991; **325**:606-612.

148 Graham NMH, Douglas RM, Ryan P. Stress and acute respiratory infection. *Am J Epidemiol* 1986; **124**:389-401.

149 Glaser R, Friedman SB, Smyth J *et al.* The differential impact of training stress and final examination stress on herpesvirus latency at the United States Military Academy at West Point. *Brain Behav Immun* 1999; **13**:240-251.

150 Stowe RP, Pierson DL, Feeback DL, Barrett AD. Stress-induced reactivation of Epstein-Barr virus in astronauts. *Neuroimmunomodulation* 2000; **8**:51-58.

151 Esterling BA, Antoni MH, Kumar M, Schneiderman N. Emotional repression, stress disclosure responses, and Epstein-Barr viral capsid antigen titers. *Psychosom Med* 1990; **52**:397-410.

152 Cohen F, Kemeny ME, Kearney KA *et al.* Persistent stress as a predictor of genital herpes recurrence. *Arch Intern Med* 1999; **159**:2430-2436.

153 Schmader K, Studenski S, MacMillan J, Grufferman S, Cohen HJ. Are stressful life events risk factors for herpes zoster? *JAGS* 1990; **38**:1188-1194.

154 Head H, Campbell AW. The pathology of herpes zoster and its bearing on sensory localisation. *Brain* 1900; **23**:353-523.

155 Arndt V, Vine MF, Weigle K. Environmental chemical exposures and risk of zoster. *Environ Health Perspect* 1999; **107**:835-841.

156 Juel-Jensen BE. The natural history of shingles: events associated with reactivation of varicella-zoster virus. *J R Coll Gen Pract* 1970; **20**:323-327.

157 Feinstein A, Trau H, Schewach-Millet M. Herpes zoster in a husband and wife. *Int J Dermatol* 1980; **19**(9):514-516.

158 Mackenzie J. Zoster in families. *BMJ* 1974; **4**(5936):108.

159 Hope-Simpson RE. Zoster in three children in family. *BMJ* 1974; **3**:626.

160 Bukharovich AM, Kviatkovskaia GV, Kucher LN. Herpes zoster in 2 sisters. *Vestn Dermatol Venerol* 1990; **8**:62-64.

161 Thomas M. Shingles reconsidered. *Lancet* 1964; **ii**:47.

162 Thomas M, Robertson WJ. Dermal transmission of visus as a cause of shingles. *Lancet* 1971; **ii**:1349-1350.

163 Berlin BS, Campbell MD. Hospital-acquired herpes zoster following exposure to chickenpox. *JAMA* 1970; **211**:1831-1833.

164 Morens DM. Zoster after exposure to varicella-zoster virus. *JAMA* 1985; **253**:1722-1723.

165 Straus SE, Ostrove JM, Inchauspe G *et al.* Varicella-zoster virus infection: biology, natural history, treatment and prevention. *Ann Intern Med* 1988; **108**:221-237.

166 Palmer SR, Caul EO, Donald DE, Kwantes W, Tillett H. An outbreak of shingles? *Lancet* 1985; **2**:1108-1111.

167 Martinez-Martin P, Estevez E, Rapun JL, Echevarria JM, Garcia-Saiz A. Clusters of shingles. *Lancet* 1985; **2**:1433-1434.

168 Rahman M. Outbreak of chickenpox and herpes zoster in a geriatric hospital. *Br J Clin Pract* 1979; **33**:291-292.

169 Haanpaa M, Nurmikko T, Hurme M. Polymorphism of the IL-10 gene is associated with susceptibility to herpes zoster. *Scand J Infect Dis* 2002; **34**:112-114.

170 Turner DM, Williams DM, Sankaran D *et al.* An investigation of polymorphism in the interleukin-10 gene promoter. *Immunogenetics* 1997; **24**:1-8.

171 Helminen ME, Kilpinen S, Virta M, Hurme M. Susceptibility to primary Epstein Barr virus infection is associated with IL-10 gene-promoter polymorphism. *J Infect Dis* 2001; **184**:780.

172 IARC Working Group. Exposure Data. In: World Health Organisation, International Agency for Research On Cancer (Editors). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Solar and Ultraviolet Radiation. Lyon: International Agency for Research on Cancer, 1992.

173 Garssen J, Van Loveren H. Effects of ultraviolet exposure on the immune system. *Crit Rev Immunol* 2001; **21**:359-397.

174 Applegate LE, Ley RD, Alcalay J, Kripke ML. Identification of the molecular target for the suppression of contact bypersensitivity by ultraviolet radiation. *J Exp Med* 1989; **170**:1117-1131.

175 Kripke ML, Cox PA, Alas LG, Yarosh DB. Pyrimidine dimers in DNA initiate systemic immunosuppression in UV-irradiated mice. *Proc Natl Acad Sci USA* 1992; **89**:7516-7520.

176 Noonan FP, De Fabo EC. Immunosuppression by ultraviolet B radiation: initiation by urocanic acid. *Immunol Today* 1992; **13**:250-254.

177 Rivas JM, Ullrich SE. Systemic suppression of delayed-type hypersensitivity by supernatants from UV-irradiated keratinocytes. An essential role for keratinocyte-derived IL-10. *J Immunol* 1992; **149**:3865-3871.

178 Shreedhar V, Giese T, Sung VW, Ullrich SE. A cytokine cascade including prostaglandin E2, IL-4, and IL-10 is responsible for UV-induced systemic immune suppression. *J Immunol* 1998; **160**:3783-3789.

179 Moodycliffe AM, Kimber I, Norval M. Role of tumour necrosis factor-α in ultraviolet-B light induced dendritic cell migration and suppression of contact hypersensitivity. *Immunology* 1994; **81**:79-84.

180 Barr RM, Walker SL, Tsang W *et al.* Suppressed alloantigen presentation, increased TNA-α, IL-1, IL-1a, IL-10, and modulation of TNF-R in UV-irradiated human skin. *J Invest Dermatol* 1999; **112**:692-698.

181 Duthie MS, Kimber I, Dearman RI, Norval M. Differential effects of UV-A1 and UV-B radiation on Langerhans cell migration in mice. *J Photochem Photobiol B* 2000; **57**:123-131.

182 De Gruijl FR, Both H, van Weelden H *et al.* Epidermal Langerhans cell depletion after artificial ultraviolet B irradiation of human skin *in vivo*: apoptosis *versus* migration. *J Invest Dermatol* 2002; **118**:812-817.

183 Simon C, Tigelaar RE, Bergstresser PR, Edelbaum D, Cruz PD. Ultraviolet B radiation converts Langerhans cells from immunogenic to tolerogenic antigen-presenting cells: induction of specific clonal anergy in CD4+ T helper 1 cells. *J Immunol* 1991; **146**:485-491.

184 Cooper KD, Neises GR, Katz SI. Antigen-presenting OKM5+ melanophages appear in human epidermis after ultraviolet radiation. *J Invest Dermatol* 1986; **86**:363-370.

185 Baadsgaard O, Salvo B, Mannie A *et al.* In vivo ultraviolet-exposed human epidermal cells activate T suppressor cell pathways that involve CD4+CD45RA+ suppressor-inducer T cells. *J Immunol* 1990; **145**:2854-2861.

186 Kang K, Hammerberg C, Meunier L, Cooper KD. CD11b+ macrophages that infiltrate human epidermis after in vivo ultraviolet exposure potently produce IL-10 and represent the major secretory source of epidermal IL-10 protein. *J Immunol* 1994; **153**:5256-5264.

187 Morison WL. Phototherapy and Photochemotherapy of Skin Disease. New York: Praeger, 1983.

188 Clydesdale GJ, Dandie GW, Muller HK. Ultraviolet light induced injury: immunological and inflammatory effects. *Immunol Cell Biol* 2001; **79**:547-568.

189 Hersey P, Haran G, Hasic E, Edwards A. Alteration of T cell subsets and induction of suppressor T cell activity in normal subjects after exposure to sunlight. *J Immunol* 1983; **31**:171-174.

190 Falkenbach A, Sedlmeyer A. Travel to sunny countries is associated with changes in immunological parameters. *Photodermatol Photoimmunol Photomed* 1997; **13**:139-142.

191 Kanariou M, Petridou E, Vrachnou E, Trichopoulos D. Lymphocyte alterations after prolonged sunlight exposure. *J Epidemiol Biostat* 2001; **6**:463-465.

192 Morison WL, Parrish JA, Bloch KJ, Krugler JI. *In vivo* effect of UV-B on lymphocyte function. *Br J Dermatol* 1979; **101**:513-519.

193 Kelly DA, Young AR, McGregor JM *et al.* Sensitivity to sunburn is associated with susceptibility to ultraviolet radiation-induced suppression of cutaneous cell-mediated immunity. *J Exp Med* 2000; **191**:561-566.

194 Kalimo K, Koulu KL, Jansen CT. Effect of a single UVB or PUVA exposure on immediate and delayed skin hypersensitivity reactions in humans. *Arch Dermatol Res* 1983; **275**:374-378.

195 Räsänen L, Reunala T, Lehto M *et al.* Immediate decrease in antigen-presenting function and delayed enhancement of interleukin-1 production in human epidermal cells after *in vivo* UVB irradiation. *Br J Dermatol* 1989; **120**:589-596.

196 Yoshikawa J, Rae V, Bruins-Slot W, Van den Berg JW. Susceptibility to effects of UVB radiation on induction of contact hypersensitivity as a risk factor for skin cancer in humans. *J Invest Dermatol* 1990; **95**:530-536.

197 Cooper KD, Oberhelman BS, Hamilton MS *et al.* UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: relationship to dose, $CD1a^{-}$ DR^{+} epidermal macrophage induction and Langerhans cell depletion. *Proc Natl Acad Sci USA* 1992; **89**:8497-8501.

198 Hersey P, Bradley M, Hasic E *et al.* Immunological effects of solarium exposure. *Lancet* 1983; **i**:545-548.

199 Damian DL, Barnetson RStC, Halliday GM. Low-dose UVA and UVB have different time courses for suppression of contact hypersensitivity to a recall antigen in humans. *J Invest Dermatol* 1999; **112**:939-944.

200 Kelly DA, Walker SL, McGregor JM, Young AR. A single exposure of solar simulated radiation suppresses contact hypersensitivity responses both locally and systemically in humans: quantitative studies with high-frequency ultrasound. *J Photochem Photobiol B* 1998; **44**:130-142.

201 Cestari TF, Kripke ML, Baptista PL, Bakos L, Bucana CD. Ultraviolet radiation decreases the granulomatous response to lepromin in humans. *J Invest Dermatol* 1995; **105**:8-13.

202 Sleijffers A, Garssen J, De Gruijl F *et al.* Influence of ultraviolet B exposure on immune responses following hepatitis B vaccination in human volunteers. *J Invest Dermatol* 2001; **117**:1144-1150.

203 Miura S, Kulka M, Smith CC *et al.* Cutaneous ultraviolet radiation inhibits herpes simplex virus-induced lymphoproliferation in latently infected subjects. *Clin Immunol Immunopathol* 1994; **72**:62-69.

204 Hersey P, MacDonald M, Burns C *et al.* Analysis of the effect of a sunscreen agent on the suppression of natural killer cell activity induced in human subjects by radiation from solarium lamps. *J Invest Dermatol* 1987; **88**:271-276.

205 Sjovall P, Christensen OB. Local and systemic effect of ultraviolet irradiation (UVB and UVA) on human and allergic contact dermatitis. *Acta Dermatol* 1986; **66**:290-294.

206 McGrath H, Wilson WA, Scopelitis E. Acute effects of low-fluence ultraviolet light on human T-lymphocyte subsets. *Photochem Photobiol* 1986; **43**:627-631.

207 Matsuoka LY, McConnachie P, Wortsman J, Holick MF. Immunological responses to ultraviolet light B radiation in black individuals. *Life Sci* 1999; **64**:1563-1569.

208 Gilmour JW, Vestey JP, Norval M. The effect of UV therapy on immune function in patients with psoriasis. *Br J Dermatol* 1993; **129**:28-38.

209 Di Nuzzo S, de Rie MA, van der Loos CM, Bos JD, Teunissen MBM. Solar-simulated ultraviolet irradiation induces selective influx of CD4+ T lymphocytes in normal human skin. *Photochem Photobiol* 1996; **64**:988-993.

210 Handel-Fernandez ME, Kurimoto I, Streilein JW, Vincek V. Genetic mapping and physical cloning of UVB susceptibility region in mice. *J Invest Dermatol* 1999; **113**:224-229.

211 Vermeer M, Schmieder GJ, Yoshikawa T *et al.* Effects of ultraviolet B light on cutaneous immune responses of humans with deeply pigmented skin. *J Invest Dermatol* 1991; **97**:729-734.

212 Oberhelman LJ, Koren H, LeVee G, Cooper KD. Dose response relationships of immunosuppression by UV radiation in dark- *versus* light-skinned human subjects. *Clin Res* 1994; **42**:227A. [Abstract]

213 Tseng C, Hoffman B, Kurimoto I *et al.* Analysis of effects of ultraviolet B radiation on induction of primary allergic reactions. *J Invest Dermatol* 1992; **98**:871-875.

214 Chapman RS, Cooper KD, De Fabo EC *et al.* Solar Ultraviolet radiation and the risk of infectious disease: summary of a workshop. *Photochem Photobiol* 1995; **61**:223-247.

215 Czernielewski JM, Masouye I, Pisani A *et al.* Effect of chronic sun exposure on human Langerhans cell densities. *Photodermatology* 1988; **5**:116-120.

216 O'Dell BL, Jessen T, Becker LE, Jackson RT, Smith EB. Diminished immune response in sun-damaged skin. *Arch Dermatol* 1980; **116**:559-561.

217 Robinson JK, Rademaker AW. Relative importance of prior basal cell carcinomas, continuing sun exposure, and circulating T lymphocytes on the development of basal cell carcinoma. *J Invest Dermatol* 1992; **99**:227-231.

218 Saah AJ, Horn TD, Hoöver DR *et al.* Solar ultraviolet radiation exposure does not appear to exacerbate HIV infection in homosexual men. *AIDS* 1997; **11**:1773-1778.

219 Maas J, Termorshuizen F, Geskus RB, Coutinho RA, Van Loveren H. No indications for an ultraviolet B radiation induced decline of immunological parameters after sunlight exposure among HIV infected homosexual men. RIVM report no 640300.002. Bilthoven: RIVM, 2000.

220 Goettsch W, Garssen J, De Gruijl F, Van Loveren H. UVB-induced decreased resistance to Trichinella spiralis in the rat is related to impaired cellular immunity. *Photochem Photobiol* 1996; **64**:581-585.

221 Noonan FP, Lewis RA. UVB-induced immune suppression and infection with Schistosoma mansoni. *Photochem Photobiol* 1995; **61**:99-105.

222 Giannini SH. Effects of ultraviolet irradiation on cutaneous leishmaniasis. *Parasitol Today* 1992; **8**:44-48.

223 Norval M, el Ghorr AA. UV radiation and mouse models of herpes simplex virus infection. *Photochem Photobiol* 1996; **64**:242-245.

224 Jeevan A, Evans R, Brown EL, Kripke ML. Effect of local ultraviolet irradiation on infections of mice with *Candida albicans, Mycobacterium bovis* BCG, and *Schistosoma mansoni. J Invest Dermatol* 1992; **99**:59-64.

225 Goettsch W, Garssen J, de Klerk A *et al.* Effects of ultraviolet-B exposure on the resistance to *Listeria monocytogenes* in the rat. *Photochem Photobiol* 1996; **63**:672-679.

226 Jeevan A, Gilliam K, Heard H, Kripke ML. Effects of ultraviolet radiation on the pathogenesis of *Mycobacterium lepraemurium* infection in mice. *Exp Dermatol* 1992; **1**:152-160.

227 Vogel J, Cepeda M, Tschachler E, Napolitano LA, Jay G. UV activation of human immunodeficiency virus gene expression in transgenic mice. *J Virol* 1992; **66**:1-5.

228 Goettsch W, Garssen J, De Gruijl FR, Van Loveren H. Effects of UV-B on the resistance against infectious diseases. *Toxicol Lett* 1994; **72**:359-363.

229 El-Ghorr AA, Norval M. The effect of UV-B irradiation on secondary epidermal infection of mice with herpes simplex virus type 1. *J Gen Virol* 1996; **77**:485-491.

230 Wilson ME, Fineberg HV, Colditz GA. Geographic latitude and the efficacy of Bacillus Calmette-Guerin vaccine. *Clin Infect Dis* 1995; **20**:982-91.

231 Schein OD, Vicencio C, Munoz B *et al.* Ocular and dermatologic health effects of ultraviolet radiation exposure from the ozone hole in Southern Chile. *Am J Publ Health* 1995; **85**:546-50.

232 Termorshuizen F, Wijga A, Garssen J *et al.* Exposure to solar ultraviolet radiation in young Dutch children: assessment by means of a 6-week retrospective questionnaire. *J Exp Anal Environ Epidemiol* 2002; **12**:204-213.

233 Bouwes Bavinck JN, de Boer A, Vermeer BJ *et al.* Sunlight, keratotic skin lesions and skin cancer in renal transplant recipients. *Br J Dermatol* 1993; **129**:242-249.

234 Perna JJ, Mannix ML, Rooney JF, Notkins AL, Straus SE. Reactivation of latent herpes simplex virus infection by ultraviolet light: a human model. *J Am Acad Dermatol* 1987; **17**:473-478.

235 Rooney JF, Bryson Y, Mannix ML *et al.* Prevention of ultraviolet-light-induced herpes labialis by sunscreen. *Lancet* 1991; **338**:1419-1422.

236 Spruance SL. Pathogenesis of herpes simpex labialis: experimental induction of lesions with UV light. *J Clin Microbiol* 1985; **22**:366-368.

237 Klein KL, Linnemann CC. Induction of recurrent genital herpex simplex virus type 2 infection by ultraviolet light. *Lancet* 1986; **i**:796-797.

238 Friedman A, Halevy O, Schrift M, Arazi Y, Sklan D. Retinoic acid promotes proliferation and induces expression of retinoic acid receptor-alpha gene in murine T lymphocytes. *Cell Immunol* 1993; **152**:240-248.

239 Ross AC. Vitamin A status: relationship to immunity and the antibody response. *PSEBM* 1992; **200**:303-320.

240 Rall LC, Meydani SN. Vitamin B6 and immune competence. *Nutr Rev* 1993; **51**:217-225.

241 Brennan LA, Morris GM, Wasson GR, Hannigan BM, Barnett YA. The effect of vitamin C or vitamin E supplementation on basal and H202-induced DNA damage in human lymphocytes. *Br J Nutr* 2000; **84**:195-202.

242 Campbell JD, Cole M, Bunditrutavorn B, Vell AT. Ascorbic acid is a potent inhibitor of various forms of T cell apoptosis. *Cell Immunol* 1999; **194**:1-5.

243 Beharka A, Redican S, Leka L, Meydani SN. Vitamin E status and immune function. *Methods Enzymol* 1997; **282**:247-263.

244 Beisel WR. Single nutrients and immunity. *Am J Clin Nutr* 1982; **35**:417-468.

245 Prasad AS, Meftah S, Abdallah J *et al.* Serum thymulin in human zinc deficiency. *J Clin Invest* 1988; **82**:1202-1210.

246 Fraker PJ, King LE, Laako T, Vollmer TL. The dynamic link between the integrity of the immune system and zinc status. *J Nutr* 2000; **130**:1399-1406S.

247 Beck FWJ, Prasad AS, Kaplan J, Fitzgerald JT, Brewer GJ. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-decifient humans. *Am J Physiol* 1997; **272**:E1002-1007.

248 Brock JH, Mulero V. Cellular and molecular aspects of iron and immune function. *Proc Nutr Soc* 2000; **59**:537-540.

249 Working Group on the Nutrition of Elderly People, the Committee on Medical Aspects of Food Policy. The Nutrition of Elderly People. London: HMSO, 1992.

250 Ferro-Luzzi A, Maiani G, Scaccini C. Nutritional vulnerability of the Italian elderly: Facts and figures. In: Lintas C, Spadoni MA (Editors). Food Safety and Health Protection (monograph no.28). Rome: CNR/IPRA, 1988.

251 Nutrition in the elderly: the Boston nutritional status survey. London: Smith-Gordon, 1990.

252 Prasad AS, Fitzgerald JT, Hess JW *et al.* Zinc deficiency in elderly patients. *Clin Geriatr Med* 1995; **11**:567-576.

253 Azais-Braesco V, Moriniere C, Guesne B *et al.* Vitamin A status in the institutionalized elderly. Critical analysis of four evaluation criteria: dietary vitamin A intake, serum retinol, relative dose-response test (RDR) and impression cytology with transfer (ICT). *Int J Vitam Nutr Res* 1995; **65**:161.

254 Russell RM. New views on the RDAs for older adults. *J Am Diet Assoc* 1997; **97**:515-518.

255 Chavance M, Herbeth B, Fournier C, Janot C, Vernhes G. Vitamin status, immunity and infections in an elderly population. *Eur J Clin Nutr* 1989; **43**:827-835.

256 Meydani SN, Ribaya-Mercado JD, Russell RM *et al.* Vitamin B-6 deficiency impairs interleukin 2 production and lymphocyte proliferation in elderly adults. *Am J Clin Nutr* 1991; **53**:1275-1280.

257 Prasad AS, Fitzgerald JT, Hess JW *et al.* Zinc deficiency in elderly patients. *Nutrition* 1993; **9**:218-224.

258 Roebothan BV, Chandra RK. Relationship between nutritional status and immune function of elderly people. *Age Ageing* 1994; **23**:49-53.

259 Penn ND, Purkins L, Kelleher J *et al.* The effect of dietary supplementation with vitamins A, C and E on cell-mediated immune function in elderly long-stay patients: a randomized controlled trial. *Age Ageing* 1991; **20**:169-174.

260 Payette H, Rola-Pleszczynski M, Ghadirian P. Nutrition factors in relation to cellular and regulatory immune variables in a free-living elderly population. *Am J Clin Nutr* 1990; **52**:927-932.

261 Mocchegiani E, Muzzioli M, Gaetti R *et al.* Contribution of zinc to reduce CD4+ risk factor for 'severe' infection relapse in aging: parallelism with HIV. *Int J Immunopharmacol* 1999; **21**:271-281.

262 Chandra RK. Nutrition and immunity in the elderly: clinical significance. *Nutr Rev* 1995; **53**:S80-85.

263 Bogden JD, Oleske JM, Lavenhar MA *et al.* Zinc and immunocompetence in elderly people: effects of zinc supplementation for 3 months. *Am J Clin Nutr* 1988; **48**:655-663.

264 Bogden JD, Oleske JM, Lavenhar MA *et al.* Effects of one year of supplementation with zinc and other micronutrients on cellular immunity in the elderly. *J Am Coll Nutr* 1990; **9**:214-225.

265 Kennes B, Dumont I, Brohee D, Hubert C, Neve P. Effect of vitamin C supplementation on cell-mediated immunity in old people. *Gerontology* 1983; **29**:305-310.

266 Meydani SN, Barklund MP, Liu S *et al.* Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *Am J Clin Nutr* 1990; **52**:557-563.

267 Meydani SN, Meydani M, Blumberg JB *et al.* Vitamin E supplementation and in vivo immune response in healthy elderly subjects: a randomised controlled trial. *JAMA* 1997; **277**:1380-1386.

268 Pallast EG, Schouten EG, de Waart FG *et al.* Effect of 50- and 100-mg vitamin E supplements on cellular immune function in noninstitutionalized elderly persons. *Am J Clin Nutr* 1999; **69**:1273-1281.

269 Fortes C, Forastiere F, Agabiti N *et al.* The effect of zinc and vitamin A supplementation on immune response in an older population. *JAGS* 1998; **46**:19-26.

270 Bogden JD, Bendich A, Kemp FW *et al.* Daily micronutrient supplements enhance delayed-hypersensitivity skin test responses in older people. *Am J Clin Nutr* 1994; **60**:437-447.

271 Boardley D, Fahlman M. Micronutrient supplementation does not attenuate seasonal decline of immune system indexes in well-nourished elderly women: a placebo-controlled study. *J Am Diet Assoc* 2000; **100**:356-359.

272 Chandra RK. Effect of vitamin and trace-element supplementation on immune responses and infection in elderly subjects. *Lancet* 1992; **340**:1124-1127.

273 Chandra RK. Excessive intake of zinc impairs immune responses. *JAMA* 1984; **252**:1443-1446.

274 Prasad JS. Effect of vitamin E supplementation on leukocyte function. *Am J Clin Nutr* 1980; **33**:606-608.

275 Walker Jr. EM, Walker SM. Effects of iron overload on the immune system. *Ann Clin Lab Sci* 2000; **30**:354-365.

276 Girodon F, Lombard M, Galan P *et al.* Effect of micronutrient supplementation on infection in institutionalized elderly subjects: a controlled trial. *Ann Nutr Metab* 1997; **41**:98-107.

277 Chavance M, Herbeth B, Lemoine A, Zhu B-P. Does multivitamin supplementation prevent infections in healthy elderly subjects? A controlled trial. *Int J Vitam Nutr Res* 1993; **63**:11-16.

278 Murphy S, West KP, Greenough WB *et al.* Impact of vitamin A supplementation on the incidence of infection in elderly nursing-home residents: a randomized controlled trial. *Age Ageing* 1992; **21**:435-439.

279 Population Estimates Unit, Office for National Statistics. Resident Population Estimates for Local Authorities in England, mid 1998. Oxford: HMSO, 1999.

280 Department of Transport, Local Government and the Regions. Measuring Multiple Deprivation at the Small Area Level: The Indices of Deprivation 2000. London: DTLR, 2000.

281 Office of Population Censuses and Surveys. Census of England and Wales 1991: County Reports - Inner London (Vol 1). London, HMSO, 1993.

282 Schlesselman JJ. Case-Control Studies: Design, Conduct, Analysis. New York: Oxford University Press, 1982.

283 Schmader K, George LK, Newton R, Hamilton JD. The accuracy of self-report of herpes zoster. *J Clin Epidemiol* 1994; **47**:1271-1276.

284 Unlinked Anonymous Surveys Steering Group. Prevalence of HIV and hepatitis infections in the United Kingdom 1999. London: Department of Health, Public Health Laboratory Service, Institute of Child Health (London), Scottish Centre for Infection and Environmental Health, 2000.

285 Weller TH. Varicella-herpes zoster virus. In: Evans AS (Editor). Viral Infections of Humans: Epidemiology and Control. New York: Plenum Medical Book Company, 1997.

286 Kricker A, Armstrong BK, Jones ME, Burton RC. Measurement of behaviours influencing exposure to solar UV radiation. In: Health, Solar UV Radiation and Environmental Change. Lyon: International Agency for Research on Cancer, 1993.

287 Kricker A, Armstrong BK, English DR, Heenan PJ. A dose-response curve for sun exposure and basal cell carcinoma. *Int J Cancer* 1995; **60**:482-488.

288 Willett W. Food-Frequency Methods. In: Willett W (Editor). Nutritional Epidemiology. Oxford: OUP, 1998.

289 Willett W. Nature of Variation in Diet. In: Willett W (Editor). Nutritional Epidemiology. Oxford: OUP, 1998.

290 Willett W, Lenart E. Reproducibility and Validity of Food-Frequency Questionnaires. In: Willett W (Editor). Nutritional Epidemiology. Oxford: OUP, 1998.

291 Willett WC, Sampson L, Stampfer MJ *et al.* Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985; **122**:51-65.

292 Willett W, Sampson L, Brown ML *et al.* The use of a self administered questionnaire to assess diet four years in the past. *Am J Epidemiol* 1988; **127**:188-199.

293 Guthrie HA. Selection and quantification of typical food portions by young adults. *J Am Diet Assoc* 1984; **84**:1444.

294 Smith AF, Jobe JB, Mingay DJ. Question-induced cognitive biases in reports of dietary intake by college men and women. *Health Psychol* 1991; **10**:244-251.

295 Hunter DJ, Sampson L, Stampfer MJ *et al.* Variability in portion sizes of commonly consumed foods among a population of women in the United States. *Am J Epidemiol* 1988; **127**:1240-1249.

296 Heady JA. Diets of bank clerks. Development of a method of classifying the diets of individuals for use in epidemiologic studies. *J R Statist Soc* 1961; **124**: 336-361.

297 Samet JM, Humble CG, Skipper BE. Alternatives in the collection and analysis of food frequency interview data. *Am J Epidemiol* 1984; **120**:572-581.

298 Hernandez-Avila M, Master C, Hunter DJ, Buring J, Philips J, Willett WC. Influence of additional portion size data on the validity of a semi-quantitative food frequency questionnaire. *Am J Epidemiol* 1988; **128**: 891 [Abstract].

299 Willett WC, Reynolds RD, Cottrell-Hoehner S, Sampson L, Browne ML. Validation of a semi-quantitative food frequency questionnaire: Comparison with a 1-year diet record. *J Am Diet Assoc* 1987; **87**:43-47.

300 Rimm EB, Giovannucci EL, Stampfer MJ *et al.* Reproducibility and validity of and expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol* 1992; **135**:1114-1126.

301 Jacques PF, Sulsky SI, Sadowski JA *et al.* Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. *Am J Clin Nutr* 1993; **57**:182-189.

302 Vioque J, Gonzalez L. Validity of a food frequency questionnaire (preliminary results). *Eur J Cancer* 1991; **1**:19.

303 Mannisto S, Virtanen M, Mikkonen T, Pietinen P. Reproducibility and validity of a food frequency questionnaire in a case-control study on breast cancer. *J Clin Epidemiol* 1996; **49**:401-409.

304 Bingham SA, Gill C, Welch A *et al.* Validation of dietary assessment methods in the UK arm of EPIC using weighed records, and 24-hour urinary nitrogen and potassium and serum vitamin C and carotenoids as biomarkers. *Int J Epidemiol* 1997; **26** (Suppl 1):S137-151.

305 Katsouyanni K, Rimm EB, Gnardellis C *et al.* Reproducibility and relative validity of an extensive semi-quantitative food frequency questionnaire using dietary records and biochemical markers among Greek schoolteachers. *Int J Epidemiol* 1997; **26** (Suppl. 1):S118-127.

306 Subar AF, Thompson FC, Kipnis V *et al.* Comparative validation of the Block, Willett and National Cancer Institute food frequency questionnaires. *Am J Epidemiol* 2001; **154** :1089-1099.

307 Bingham SA, Day NE. Using biochemical markers to assess the validity of prospective dietary assessment methods and the effect of energy adjustment. *Am J Clin Nutr* 1997; **65** (Suppl.):1130S-1137S.

308 McKeown NM, Day NE, Welch AA *et al.* Use of biological markers to validate self-reported dietary intake in a random sample of the European Prospective Investigation into Cancer United Kingdom Norfolk cohort. *Am J Clin Nutr* 2001; **74**:188-196.

309 Kaaks R, Slimani N, Riboli E. Pilot phase studies on the accuracy of dietary intake measurements in the EPIC Project: overall evaluation of results. *Int J Epidemiol* 1997; **26** (Suppl 1):S26-36.

310 Bingham SA, Welch AA, McTaggart A *et al.* Nutritional methods in the European prospective investigation of cancer in Norfolk. *Public Health Nutr* 2001; **4**:847-858.

311 Cook RT. Alcohol abuse, alcoholism, and damage to the immune system - a review. *Alcohol Clin Exp Res* 1998; **22**:1927-1942.

312 Schleifer SJ, Benton T, Keller SE, Dhaibar Y. Immune measures in alcohol-dependent persons with minor health abnormalities. *Alcohol* 2002; **26**:35-41.

313 Smart YC, Cox J, Roberts TK, Brinsmead MW, Burton RC. Differential effect of cigarette smoking on recirculating T lymphocyte subsets in pregnant women. *J Immunol* 1986; **137**:1-3.

314 Moszczynski P, Zabinski Z, Moszczynski PJ *et al.* Immunological findings in cigarette smokers. *Toxicol Lett* 2001; **118**:121-127.

315 Kiyak A, Liang J, Kahana E. The geriatric scale of recent life events. In: Mangen DJ, Peterson WA (Editors). Research Instruments in Social Gerontology. Minneapolis: University of Minnesota Press, 1982.

316 Hawrami K, Breuer J. Development of a flurogenic polymerase chain reaction assay (Taqman) for the detection and quantitation of varicella zoster virus. *J Virol Meth* 1999; **79**:33-40.

317 The Times Atlas of the World: 9th Comprehensive Edition. London: Harper Collins, 1994.

318 Henning D. Atlas of the Surface Heat Balance of the Continents. Berlin: Gebrüder Borntraeger, 1989.

319 Epi Info: a word processing, database and statistics program for epidemiology on microcomputers. Atlanta,GA: Centers for Disease Control and Prevention, 1994.

320 Stata Statistical Software. College Station, TX: Stata Corporation, 1999.

321 Maldonado G, Greenland S. Simulation study of confounder-selection strategies. *Am J Epidemiol* 1993; **138**:923-936.

322 Kricker A, Armstrong BK, Jones ME, Burton RC. Measurement of solar UV irradiance at the surface of the Earth. In: Health, Solar UV Radiation and Environmental Change. Lyon: International Agency for Research on Cancer, 1993.

323 Bjorn LO. Computer Programs for Estimating Ultraviolet Radiation in Daylight. In: Diffey BL (Editor). Radiation Measurement in Photobiology. London: Academic Press, 1989.

324 Green AES, Sawada T, Shettle EP. The middle ultraviolet reaching the ground. *Photochem Photobiol* 1974; **19**:251-259.

325 Green AES, Cross KR, Smith LA. Improved analytic characterization of ultraviolet skylight. *Photochem Photobiol* 1980; **31**:59-65.

326 Green AES. The penetration of ultraviolet radiation to the ground. *Physiol Plant* 1983; **58** :351-359.

327 Roy CR, Gies HP. Solar Ultraviolet Radiation: Measurement Techniques and the Australian Network. In: Ilyas M (Editor). Ozone Depletion: Implications for the Tropics. Penang: University of Science Malaysia & UNEP, 1993.

328 Diffey BL, Elwood JM. Tables of Ambient Solar Ultraviolet Radiation for Use in Epidemiological Studies of Malignant Melanoma and Other Diseases. In: Gallagher R, Elwood JM (Editors). Epidemiological Aspects of Cutaneous Malignant Melanoma. New York: Kluwer, 1994.

329 Crawley H. Food Portion Sizes. 2nd Ed. London: HMSO, 1993.

330 Widdowson EM, McCance RA, Holland B. McCance and Widdowsons's The Composition of Foods. 5th Revised and Extended Edition. Cambridge: Royal Soc Chem & MAFF, 1991.

331 Holland B, Unwin ID, Buss DH. McCance & Widdowson's The Composition of Foods: Cereals and Cereal Products. 4th Edition (Supplement 3). Cambridge: Royal Soc Chem, 1988.

332 Holland B, Brown J, Buss DH. McCance and Widdowsons's The Composition of Foods: Fish and Fish Products. 5th Revised and Extended Edition (3rd Supplement). Cambridge: Royal Soc Chem & MAFF, 1993.

333 Chan W, Brown J, Church SM. McCance and Widdowsons's The Composition of Foods: Meat Products and Dishes. 5th Revised and Extended Edition (6th Supplement). Cambridge: Royal Soc Chem, 1996.

334 Chan W, Brown J, Lee SM. McCance and Widdowsons's The Composition of Foods: Meat, Poultry and Game. 5th Revised and Extended Edition (5th Supplement). Cambridge: Royal Soc Chem, 1995.

335 Chan W, McCance RA, Buss DH, Brown J. McCance and Widdowsons's The Composition of Foods: Miscellaneous Foods. 5th Revised and Extended Edition (4th Supplement). Cambridge: Royal Soc Chem and MAFF, 1994.

336 Holland B, Unwin ID, Buss DH. McCance and Widdowsons's The Composition of Foods: Fruit and Nuts. 5th Revised and Extended Edition (1st Supplement). Cambridge: Royal Soc Chem, 1992.

337 McCance RA, Widdowson EM, Holland B, Welsh AA, Buss DH. McCance and Widdowsons's The Composition of Foods: Vegetable Dishes. 5th Revised and Extended Edition (2nd Supplement). Cambridge: Royal Soc Chem, 1992.

338 Holland B, Unwin ID, Buss DH. McCance and Widdowsons's The Composition of Foods: Vegetables, Herbs and Spices. 4th Edition (5th Supplement). Cambridge: Royal Soc Chem, 1991.

339 Holland B, Unwin ID, Buss DH. McCance and Widdowsons's The Composition of Foods: Vegetables, Milk Products and Eggs. 4th Edition (4th Supplement). Cambridge: Royal Soc Chem, 1989.

340 Tan SP, Wenlock RW, Buss DH. McCance and Widdowsons's The Composition of Foods: Immigrant Foods. 4th Edition (2nd Supplement). London: HMSO, 1985.

341 Gregory J, Foster K, Tyler H, Wiseman M. The Dietary and Nutritional Survey of British Adults. London: HMSO, 1990.

342 Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G *et al.* National Diet and Nutrition Survey: people aged 65 years and over. Report of the diet and nutrition survey (Volume 1). London: The Stationery Office, 1998.

343 Social Survey Division OPCS, Ministry of Agriculture Fisheries and Food, Department of Health. Dietary and Nutritional Survey of British Adults, 1986-1987 (Study no. 2836). 1991. Colchester, ESCR Data Archive.

344 Social and Community Planning Research, University College London Dept of Epidemiology and Public Health, Medical Research Council Dunn Nutrition Unit. National Diet and Nutrition Survey of people aged 65 years and over (Study no. 4036). 1999. Colchester, ESCR Data Archive.

345 Integrated Dietary Analysis. London: IDA Publications Ltd, 1997.

346 Willett W, Stampfer M. Implications of total energy intake for epidemiologic analyses. In: Willett W (Editor). Nutritional Epidemiology. Oxford: OUP, 1998: 273-301.

347 Sethi D, Wheeler J, Rodrigues LC, Fox S, Roderick P. Investigation of under-ascertainment in epidemiological studies based in general practice. *Int J Epidemiol* 1999; **28**:106-112.

348 Taylor JR, Schmieder GJ, Shimizu T, Tie C, Streilein JW. Interrelationship between ultraviolet light and recurrent herpes simplex infections in man. *J Dermatol Sci* 1994; **8**:224-232.

349 Victora CG, Huttly SR, Fuchs SC, Olinto MT. The role of conceptual frameworks in epidemiological analysis: a hierarchical approach. *Int J Epidemiol* 1997; **26**:224-227.

350 Brisson M, Gay NJ, Edmunds WJ, Andrews NJ. Exposure to varicella boosts immunity to herpes-zoster: implications for mass vaccination against chickenpox. *Vaccine* 2002; **20**:2500-2507.

351 Thomas SL, Wheeler JG, Hall AJ. Contacts with varicella or with children and protection against herpes zoster in adults: a case-control study. *Lancet* 2002; **360**:678-682.

352 Gallagher J, Quaid B, Cryan B. Susceptibility to varicella zoster virus infection in health care workers. *Occup Med* 1996; **46**:289-292.

353 Anon. British National Formulary Number 44 (September 2002). London: British Medical Association and the Royal Pharmaceutical Society, 2002.

354 Block G, Norkus E, Hudes M, Mandel S, Helzlsouer K. Which plasma antioxidants are most related to fruit and vegetable consumption? *Am J Epidemiol* 2001; **154**:1113-1118.

355 Bajekal M, Primatesta P, Prior G (Editors). HSE 2001 - Fruit and vegetable consumption. London: The Stationery Office, 2003.

356 Hoffman-Goetz L, Klarlund Pedersen B. Exercise and the immune system: a model of the stress response? *Immunol Today* 1994; **15**:382-387.

357 Spruance SL, Freeman DJ, Stewart JC *et al.* The natural history of ultraviolt radiation-induced herpes simplex labialis and response to therapy with peroral and topical formulations of acyclovir. *J Infect Dis* 1991; **163**:728-734.

358 Adam J. Sun-protective clothing. *J Cutan Med Surg* 1998; **3**:50-53.

359 Parisi AV, Kimlin MG, Mulheran L, Meldrum LR, Randall C. Field-based measurements of personal erythemal ultraviolet exposure through a common summer garment. *Photodermatol Photoimmunol Photomed* 2000; **16**:134-138.

360 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; **299**:1259-1260.

361 Shaheen SO, Aaby P, Hall AJ *et al.* Measles and atopy in Guinea-Bissau. *Lancet* 1996; **347**:1792-1796.

362 Illi S, von Mutius E, Lau S *et al.* Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ* 2001; **322**:390-395.

363 Aaby P, Shaheen SO, Heyes CB *et al.* Early BCG vaccination and reduction in atopy in Guinea-Bissau. *Clin Exp Allergy* 2000; **30**:644-650.

364 Braun-Fahrlander C, Riedler J, Herz U *et al.* Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; **347**:869-877.

365 Nakazawa H, English D, Randell PL *et al.* UV and skin cancer specific p53 gene mutation in normal skin as a biologically relevant exposure measure. *Proc Natl Acad Sci USA* 1994; **91**:360-364.

366 Ziegler A, Leffell DJ, Kunala S *et al.* Mutation hotspots due to sunlight in the *p*53 gene of nonmelanoma skin cancers. *Proc Natl Acad Sci USA* 1993; **90**:4216-4220.

367 Bailey MH, McKinney P. Herpes zoster as a complication of a face lift. *Aesthetic Plast Surg* 1988; **12**:23-24.

368 Furuta Y, Ohtani F, Fukuda S, Inuyama Y, Nagashima K. Reactivation of varicella-zoster virus in delayed facial palsy after dental treatment and oro-facial surgery. *J Med Virol* 2000; **62**:42-45.

369 Rothman KJ, Greenland S. Modern Epidemiology (2nd Edition). Philadelphia: Lippincott-Raven, 1998.

370 Seward JF, Jumaan AO, Galil K, Wharton M. Varicella vaccine and shingles. *JAMA* 2002; **287**:2211-2212.

371 World Health Organisation. Diet, Nutrition and the Prevention of Chronic Diseases. Technical Report Series 797. Geneva: WHO, 1990.

372 Department of Health. The NHS Plan. Improving Health and Reducing Inequality. Section 1320. London: The Stationery Office, 2001.

373 Stables GJ, Subar AF, Patterson BH *et al.* Changes in vegetable and fruit consumption and awareness among US adults: results of the 1991 and 1997 5 A Day for Better Health Program surveys. *J Am Diet Assoc* 2002; **102**:809-817.

374 Department of Health. Health Effects of Climate Change in the UK. London: Department of Health, 2002.

375 Kricker A, Armstrong BK, Jones ME, Burton RC. Effects of solar UV radiation on the immune system. In: Health, Solar UV Radiation and Environmental Change. Lyon: International Agency for Research on Cancer, 1993: 81-90.

376 Committee of the Health Council of the Netherlands. UV radiation from sunlight. 1994/05E. The Hague: Health Council of the Netherlands, 1994.

377 Anon. Protecting children from ultraviolet radiation - information from the World Health Organization. *J Environ Health* 2001; **64**:45-46.

378 Melia J, Pendry L, Eiser JR, Harland C, Moss S. Evaluation of primary prevention initiatives for skin cancer: a review from a UK perspective. *Br J Dermatol* 2000; **143**:701-708.

379 Montague M, Borland R, Sinclair C. Slip! Slop! Slap! and SunSmart, 1980-2000. Skin cancer control and 20 years of population-based campaigning. *Health Educ Behav* 2001; **28**:290-305.

380 Rodrigue JR. Promoting healthier behaviors, attitudes, and beliefs toward sun exposure in parents of young children. *J Consult Clin Psychol* 1996; **64**:1431-1436.

381 Oxman MN. Immunization to reduce the frequency and severity of herpes-zoster and its complications. *Neurology* 1995; **45** (Suppl 8):S41-46.

10. Appendices 1 – 8

Appendix 1: GP reporting form

Appendix 2: Study poster for consulting room

Appendix 3: Study newsletter for practices

Appendix 4: Introductory letter to cases

Appendix 5: Study information leaflet

Appendix 6: Introductory letter to controls

Appendix 7: Study questionnaire

Appendix 8: *Lancet* paper - child contact analyses

(GP reporting form)

FAX TRANSMISSION

FROM:

TO: Dr Sara Thomas
Infectious Disease Epidemiology Unit
London School of Hygiene & Tropical Medicine
Tel: 0171 927 2496
Fax: 0171 637 4314

DETAILS OF PATIENT WITH ZOSTER

Date of consultation: __ / __ / __ GP's initials .....

Patient's Name: .....

Address: .....

..... Tel: .....

Sex: Male ☐ Female ☐ Date of birth: __ / __ / __

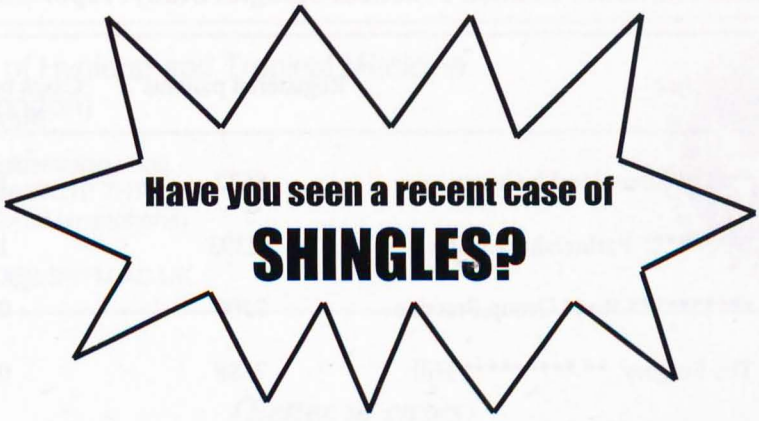
If patient refuses even to be contacted, please notify sex and date of birth ONLY

Have you just seen a case of...

Please remember to report all cases for the

London School of Hygiene & Tropical Medicine
SHINGLES STUDY
(1997-1999)

LIAISE WITH SUSAN WELLS



South London General Practices / LSHTM Shingles Study (1997-1999)

PARTICIPANTS' UPDATE - MONTH 9 (MAY 1998)

******** Health Centre: shingles cases to date**

Cases reported in May:	5
Total cases reported Sept 97-May 98:	36

List of cases ascertained in May

1.	*****	12 yrs
2.	******	67 yrs
3.	********	50 yrs
4.	*********	42 yrs
5.	*********	29 yrs

Missing cases from May?

Please let us know about any other new cases of shingles seen in May which are not listed above. These cases can still be added to the study.

A summary of all cases reported to date from the 19 participating practices is provided overleaf.

Please continue to report all shingles cases - liaise with Maureen Holder

LSHTM/South London Practices Shingles Study: reported cases, Sept 1997-May 1998

Practice	Registered patients[a]	Cases reported May 98	Total cases Sept 97-May 98
****** Street Health Centre	8532	1	9
********* Partnership (3 sites)	22393	1	21
********** Road Group Practice	7500	0	17
The Surgery, ** ********* Hill	7388	0	6
********* Health Centre	5656	1	4
********** Hill Group Practice	10465	(8)[b]	15
The Surgery, ** ******** Green	9934	3	12
******** Medical Centre	9510	0	9
The Surgery, *** ******* Way	5922	0	10
***** Hill Group Practice	15088	3	20
******* Health Centre	15449	5	36
The Surgery, ** ***** Road	9405	2	18
The *** Surgery, ***** Road	9199	1	5
********** Health Centre	7350	4	13
********* Group Practice	16314	2	26
***** ****** Partnership (2 sites)	11899	0	11
The Surgery, ** ********* Lane	6945	1	5
********** Health Centre	11884	(2)[b]	17
The Surgery, ** ********* Road	9788	0	12
TOTAL	200621	34	266

[a] Sept 1997, excluding temporary patients

[b] Including one or more case reported retrospectively

The South London General Practices / LSHTM Shingles Study has been set up to estimate the incidence of and risk factors for shingles in an urban population. All new cases of shingles are ascertained from 19 general practices, and eligible cases and controls (individuals without shingles) are also enrolled in the risk factor study.

For further information, contact your practice liaison person (listed overleaf) or Dr Sara Thomas, Infectious Disease Epidemiology Unit, London School of Hygiene & Tropical Medicine (Tel/Fax: 0171 927 2496)

London School of Hygiene and Tropical Medicine
(University of London)

Infectious Disease Epidemiology Unit
Keppel Street, London WC1E 7HT
Direct tel: 0171 927 2496 (ansaphone)
Fax: 0171 637 4314
E-MAIL ECDESTHO@LSHTM.AC.UK

(Letter to cases)

Dear ***

The *** Surgery is working with the London School of Hygiene & Tropical Medicine on a study to find out the reasons why some people get shingles. In order to do this, we need to talk to every patient from the Practice who develops shingles. I enclose a leaflet which gives details about the London School and about the study.

The Surgery has informed me that you recently developed shingles. I am therefore writing to see whether you would be able to help us with this work. As the leaflet explains, this would involve answering some questions about the places you have lived and worked, your usual diet, and recent contacts with people with chickenpox, and checking your height and weight. I would only need to visit you once, and the whole interview usually takes less than an hour and a half.

I will contact you within the next couple of days, to answer any questions you have about the study. If you decide to take part, I will then arrange to visit you at a time which is convenient to you. I hope that your shingles are not causing you too much discomfort, and I look forward to speaking with you shortly.

Yours sincerely

Dr Sara Thomas

Enc.

RISK FACTORS FOR SHINGLES (HERPES ZOSTER) IN AN URBAN POPULATION

Dr Sara Thomas
Dr Andrew Hall

INFORMATION ABOUT THE STUDY
(for people without shingles)

Infectious Disease Epidemiology Unit
London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

What is the London School?

The London School of Hygiene and Tropical Medicine is part of the University of London, and is an internationally renowned centre for teaching and research in public health. Research is carried out on a wide variety of diseases, and the findings can be used to develop ways to prevent or limit ill-health.

What is the shingles study?

Shingles (known medically as herpes zoster) is a relatively common skin condition which results in a painful, blistering skin rash. We know that it is due to the chickenpox virus which remains in our bodies following the first infection, and then for some reason becomes active again. It is not known why this happens in some people but not in others, and we are therefore carrying out a study (with the co-operation of your GP) to try to find out. We are particularly interested in whether the risk of developing shingles has anything to do with diet or sunlight exposure. We also need to know if it has anything to do with recent contact with people suffering from chickenpox. The results of this study may give us important information which will help discussions about whether to introduce chickenpox vaccination in the United Kingdom, and may also help with advice given to people about their diet and exposure to sunlight.

We have a group of people who have suffered shingles recently, but we also need to talk to people who have never had shingles as a comparison. We are therefore inviting people who have never had shingles to take part in this study.

What does the study involve?

If you have never had shingles and you decide to help us with this study, Sara Thomas (one of the study researchers) will arrange to visit you at a convenient time, to ask you some questions about the places you have lived, worked or visited (so we can estimate your exposure to sunlight during your life), your usual diet (including smoking and alcohol consumption), and any recent contacts you may have had with people with chickenpox. The interview will be completed at a single visit, and will probably take about an hour and a half. We would also like to measure your height and weight, but no other tests are needed.

You are of course not obliged to take part in this study, and you may withdraw at any time during the interview without giving us a reason. This will not affect the medical care you receive from your GP in any way. However, your participation would be greatly appreciated, as the success of the study depends on as many people taking part as possible.

Confidentiality

All information you give us remains completely confidential. Any information which we store on computer (so that we can analyse the information we collect) will be coded with a number, and not with your name. The interview forms will only be seen by the study researchers and will not be made available to anybody else.

What happens next?

We may contact you within the next few days, to talk further with you about whether you would like to help us with this research. If you have any further questions about the study, we will be happy to answer them at this time.

Thank you for your help.

SYDENHAM GREEN GROUP PRACTICE

SYDENHAM GREEN HEALTH CENTRE

26 Holmshaw Close, London SE26 4TH **Tel No.** 0181 676 8836 **Fax No.** 0171 771 4710

(GP letter to controls)

Dear

Sydenham Green Health Centre is helping the London School of Hygiene and Tropical Medicine with a study to find out the reasons why some people get shingles. In order to do this, one of their researchers would like to talk to people who have shingles, and to some people of the same age who have never had shingles. Enclosed is an information leaflet, which gives details about the London School and about their study.

Since a person your age has developed shingles, we would like to give your name to the London School as someone they might approach, to see whether you would consider helping with this work. Sara Thomas (one of the study researchers) may then contact you and discuss the study with you. If after talking to her you decide you would like to take part, she will arrange to come and see you to ask you some questions, as outlined in the information leaflet.

Unless I hear from you, I will assume that you do not mind your name being given to Dr Thomas. This does not mean that you have to take part in the study, but just that you do not mind talking with her. She will answer any questions you have about the study, so that you can then make a decision about whether you would like to participate.

If you do not wish to be contacted, please return the reply slip to Mrs Jo Bate at the Surgery.

With many thanks for your help.

Yours sincerely

Dear Mrs Bate

I do not wish to be contacted about the shingles study.

Name: Date:

Dr. B.Essex • Dr. A.Platman • Dr. J.Sikorski • Dr. S.Thomas • Dr. J.Campbell

CONFIDENTIAL
ZOSTER RISK FACTOR STUDY

Interview Date: __ / __ / __ **ID Number:** ☐☐☐☐

Zoster status: Case: ☐ (1) ⇒ a) below Control: ☐ (0) ⇒ b) below

May I begin by checking a few details about yourself?

Date of Birth: __ / __ / __ **Age:** ☐☐ **Sex:** Male ☐ (1) Female ☐ (2)

And the general practice you attend is_____ **GPID:** ☐☐

And can I check a few details about your health - starting with shingles:

a) If Case: when did the rash first appear? __ / __ /__ **Duration of rash** ☐☐ days

And which side is the rash?: Right ☐ (1) Left ☐ (2) cranial ☐ (1) cervical ☐ (2)
(note extent of rash)
thoracic ☐ (3) lumbar ☐ (4)
sacral ☐ (5) disseminated ☐ (6)
no details ☐ (9)

Was there any pain? Where was this? When did it start?
(note extent of pain)

Did your doctor give you any treatment for the shingles?
(note Rx)

Have you had shingles before? Yes ☐ (1) _____ yrs ago No ☐ (0) Don't know ☐ (9)

b) If Control: can I check that you have never had shingles? No, never ☐ (0) Yes ☐ (1)
Don't know ☐ (9)

Illnesses: and if I can check your general health: do you currently have any serious medical conditions or any serious infections? *(Also: Check if any other serious conditions in the last 5 years)*

Yes ☐ (1) ⇒ c) below No ☐ (0) Don't know ☐ (9) Decline to answer ☐ (9)

c) if Yes: do you mind telling me what these are?

Details: _____ Decline to answer ☐ (9)

_____

Current drugs: can I check with you any tablets or other treatments you are taking at the moment?

Rx (Name/dose)	How long for?

Are there any other tablets or treatments you have had in the last 12 months?

Yes ☐ (1) ⇒ What were they for, and when did you take them?_____

_____

No ☐ (0)

Residence: I would now like to check the different places you have lived during your life and the different jobs you have had, so that we can work out how much sunlight you have been exposed to at various times in your life. Do you have the residence and job calendars we sent you? Did you fill them in? (if NO, complete now)

Ethnic group What do you consider your ethnic origin to be?
(*If necessary*: ethnicity is not necessarily the same as nationality; it is how you see yourself, the origins of yourself and your family, a mixture of culture, skin colour, religion)

_____

Eligible: Yes ☐ (1) No ☐ (0)

⇒ Immunosuppressed /declined questions ☐

⇒ African origin ☐

⇒ Control with previous shingles ☐

⇒ 'Unlikely' zoster ☐

⇒ Other (state) _____

Chickenpox history: can I now ask whether you remember having chickenpox?

Yes ☐ (1) ⇒ d) No ☐ (0)

d) If Yes: How old were you? Age _____ yrs Don't know ☐ (9)

Did anyone in your household have chickenpox at the same time as you?

Yes ☐ (1) ⇒ (e) No ☐ (0) Don't know ☐ (9)

e) If Yes: how many other people got chickenpox at the same time? ☐

when did you get chickenpox in relation to the others? 1st ☐ (1) 2nd ☐ (2) 3rd+ ☐ (3) Don't know ☐ (9)

Chickenpox Contacts: Do you remember being in contact with anyone who had chickenpox or shingles in the last ten years **(since 198*)?**
(Prompts: let's start with people in the household. Has anyone in the household had chickenpox or shingles since 198? How about other family members? Friends? Anyone at work? Anyone else in the last 10 years?)*

Chickenpox: Yes ☐ (1) ⇒ (e) No ☐ (0) Shingles: Yes ☐ (2) ⇒ (e) No ☐ (0)

e) If 'Yes': who, and how long ago was that? *(Complete below)*

	How long ago?	C.pox or shingles?	TYPE OF CHICKENPOX/SHINGLES CONTACT (give details) Household member	Family/Friend (non-household)	Workplace	Other
1						
2						
3						
4						
5						
6						
7						

Child contacts: Have any of the jobs you have had in your life involved *(mark on job calendar)*

Regular contact with young children? Yes ☐ (1) No ☐ (2) Don't know ☐ (9)

Regular contact with people ill in hospital? Yes ☐ (1) No ☐ (2) Don't know ☐ (9)

Other than at work, have you been in regular contact with any young children (under 10 years old) in the last ten years? These could be members of your family, or other children.
(Prompts: lets start with any children who have lived in the household in the last 10 years. How about other children? Any family members? Friends' children? Neighbours? Anyone else? To clarify about relevant children: so this is anyone who is under 20 years old now)

Yes, household contact ☐ (1) ⇒ *(Table)* Yes, non-household contact ☐ (2) ⇒ *(Table)*

No ☐ (0) Don't know ☐ (9)

CHILD CONTACTS IN LAST 10 YEARS

	Household child contacts		Non-household child contacts		
	Who / age?	Dates (from - to)	Who / age?	Dates (from-to)	How often?
1					
2					
3					
4					
5					
6					
7					
8					

Total no. of children in household ☐ *Total no of specific children outside h/hold* ☐

Skin reaction: The next questions are about your how your skin reacts to sunlight. Firstly, how does your skin react when exposed to bright sunlight for the first time in summer. For example, if you were exposed to bright sunlight in the UK for the first time for one hour in the middle of the day, without any protection, would you:

- Get a severe sunburn with blistering? ☐ (1)
- Get painful sunburn for a few days followed by peeling? ☐ (2)
- Get mildly burnt followed by some degree of tanning? ☐ (3)
- Get darker without any burning? ☐ (4)
- None of the above (specify)_____ ☐ (5)
- Don't know ☐ (9)

What would happen if your skin was repeatedly exposed to bright sunlight in summer without protection? Would it become:

- Deeply tanned? ☐ (1)
- Moderately tanned? ☐ (2)
- Only mildly tanned because of a tendency to peel? ☐ (3)
- Only freckled, with no suntan at all? ☐ (4)
- None of the above (specify)_____ ☐ (5)
- Don't know ☐ (9)

PAST UV EXPOSURE: I would now like to find out how much time you have spent outside at various times in your life, both as part of your work and in your leisure time. So I am going to ask you about your working days and non-working days separately, and for each of these I would like you to estimate how many hours on average you spent outside between 9am and 5pm in direct light. I will ask you separately about holidays. When I say 'outside in direct light', I mean completely outside, so do not include time spent in a car or under any shade.

If we begin by considering the time when you were a child, say about 6 or 7 years old, living in *** and going to school.

In the warmer months (April to September):

- on school days, how much time did you usually spend outdoors in direct light between 9-5? ____ hrs/day

- at weekends, how much time did you usually spend outdoors in direct light between 9-5? _____ hrs/day

- during school holidays, how much time did you usually spend outdoors between 9-5? _____ hrs/day

(Prompts: did you walk to school? How long did that take? And did you go out during the day at school? How long for? What time did school finish? When you got home, did you go out to play? Was that straight away, or did you eat something first (how long did that take)? Until what time did you stay out?)

And if we think about the cooler months (October to March) when you were 6-7:

- on school days, how much time did you usually spend outdoors in direct light between 9-5? ____ hrs/day

- at weekends, how much time did you usually spend outdoors in direct light between 9-5? _____hrs/day

- during school holidays, how much time did you usually spend outdoors between 9-5? _____hrs/day

When you were a young child about this age, did you go on holiday at all?

Yes ☐(1) ⇒ (a-d) below No ☐(0) Don't know ☐

a) If YES, how often did you go on holiday during this period?

More than 1 holiday/year ☐(1)
Every year ☐(2)
One every 2-3 years ☐(3)
Every 4-5 years ☐(4)
Less frequently ☐(5)
Don't know ☐(9)

b) In which country or area did you most commonly take your holiday?

_____

c) How many days did you spend on holiday on average, during this period?_____

d) On a typical holiday, when you were a child how many hours between 9 & 5 would you usually spend outdoors in the direct light (not in the shade)?:

_____ hours

When you were outdoors, how often did you wear a hat that shaded your face from the sun?

- and how often did you wear clothes that protected you from the sun, which covered your arms and legs?

	Non-holidays		Holidays	
	Hat	Clothes	Hat	Clothes
Always or almost always (1)				
Not always but more than half the time (2)				
About half the time (3)				
Less than half the time (4)				
Never or hardly ever (5)				
Don't know (9)				

Last 20 yrs: if we now consider your exposure to sunlight later in your life (**Complete table,** last page):

(For 20 yrs ago): if we start with 20 years ago (so around 1977(8)), when you were ** years old working as a *** and living in *** , if we consider just the warmer months (April to September):

- On days you worked, how much time did you usually spend outdoors in direct light between 9-5?

- and at weekends or days off, how much time did you usually spend outdoors in direct light between 9-5?

And if we think about the cooler months (October to March) during this time in your life:

- On days you worked, how much time did you usually spend outdoors in direct light between 9-5?

- and between 9-5 at weekends or days off?

And during this period of your life did you go on holiday or take any trips during the warmer months? (Apr-Sept)?

Yes ☐ (1) ⇒ (Table) No ☐ (0) Don't know ☐ (9)

a) If YES, how often did you go on holiday during this period? (More than 1 holiday/year =(1), Every year=(2); one every 2-3 years=(3); every 4-5 years=(4); less frequently=(5); don't know = (9))

b) In which country or area did you most commonly take your holiday?

c) And on average, how long would you go on holiday for?

d) On a typical holiday, how many hours between 9 & 5 would you usually spend outdoors in the direct light (not in the shade)?

And during this period of your life, did you take any holidays during the cooler months (October to March)?

Yes ☐ (1) ⇒ (a-d) below No ☐ (0) Don't know ☐ (9)

a) If YES, how often did you go on holiday during this period? [>1 holiday/year = (1), Every year = (2); one every 2-3 years = (3); every 3-4 years; = (4) less frequently = (5); don't know = (9)]

b) In which country or area did you most commonly take your holiday?

c) How many days did you spend on holiday on average, during this period?

d) On a typical holiday, how many hours between 9 & 5 would you usually spend outdoors in the direct light not in the shade)?:

And if we consider any protection against the sun during this time: when you were outdoors at work, or on non-working days (but not holidays), how often did you wear a hat that protected your face from the sun? And how about clothes that protected your arms and legs from the sun (Always/almost always (1); not always, but more than 1/2 the time (2); about half the time (3); less than half the time (4); never, or almost never (5))

And during holidays?

REPEAT FOR 10 YEARS AGO AND DURING THE LAST YEAR - ALSO FOR OTHER RESIDENCES OUTSIDE THE UK IN THE LAST 20 YRS.

Previous jobs: Are there any other jobs where for six months or more in which you usually worked outdoors for more than one hour a day between 9-5 (Job includes periods of unemployment, unpaid jobs, and looking after the home or children)

Yes ☐ (1) No ☐ (0) Don't know ☐ (9)

If Yes: complete columns on amount of time outdoors & use of hat/protective clothing

Outdoor leisure activities: I'd like to ask about outdoor leisure activities that you may have taken part in the last 20 years.

Since you were *** years old have you done any of the following between 9-5 on at least 10 days in one year:

- Sports, such as tennis, cricket, football? Yes ☐ (1) No ☐ (0) Don't know ☐ (9)
- Golf Yes ☐ (1) No ☐ (0) Don't know ☐ (9)
- Lawn bowls Yes ☐ (1) No ☐ (0) Don't know ☐ (9)
- Fishing Yes ☐ (1) No ☐ (0) Don't know ☐ (9)
- Swimming in an outdoor swimming pool Yes ☐ (1) No ☐ (0) Don't know ☐ (9)
- Walking or jogging Yes ☐ (1) No ☐ (0) Don't know ☐ (9)
- Gardening Yes ☐ (1) No ☐ (0) Don't know ☐ (9)
- Watching outdoor sports Yes ☐ (1) No ☐ (0) Don't know ☐ (9)
- Other (specify)_____

For every activity answered as YES, fill in the table below

	TYPE OF ACTIVITY					
In what year did you start this activity?						
When did you last do this activity? (if this year, give date)						
So overall you you have done this for about ** years?						
On average, how often did you do it - during the warmer months - during the cooler months						
On average, how many hours a day between 9-5 were you outdoors (not in the shade) doing this activity each time you did it?						

In the warmer months, do you sunbathe or sit out to catch the sun (other than during holidays)?

Yes ☐ (1) No ☐ (0) Don't know ☐ (9)

If Yes: for how many years have you done this?

how often on average do you do it? _____

and when you do sunbathe, how many hours do you usually do it for? ☐☐

and when was the last time you sunbathed? _____

SUNBURN: In general, during your life have you ever been sunburned so badly so as to cause blistering?

Yes ☐ (1) ⇒ below No ☐ (0) Don't know ☐ (9)

If YES, how long ago did this happen? (*Document each occasion*)

1) _____ 4) _____

2) _____ 5) _____

3) _____ 6) _____

Are there any other times when you have been sunburned badly enough so as to cause discomfort for 2 days or more?

Yes ☐ (1) ⇒ below No ☐ (0) Don't know ☐ (9)

If YES, how long ago did this happen? (*Document each occasion*)

1) _____ 4) _____

2) _____ 5) _____

3) _____ 6) _____

SUNSCREEN: have you ever used a sunscreen lotion to prevent sunburn when out in the sun?

Yes ☐ (1) ⇒ a-d No ☐ (0) Don't know ☐ (9)

a) If YES, when did you first start using sunscreen? _____

b) And how often do you use it?	**On Holiday?**	**Otherwise?**
- Always or almost always	☐ (1)	☐ (1)
- Not always, but more than half the time	☐ (2)	☐ (2)
- More or less half the time	☐ (3)	☐ (3)
- Less than half the time	☐ (4)	☐ (4)
- Never or hardly never	☐ (5)	☐ (5)

c) Is the sunscreen you usually use high protection (ie SPF of 10 or more)?

Yes ☐ (1) No ☐ (0) Don't know ☐ (9)

d) Has your sunscreen usage changed over time, and if so, how? _____

_____

SUNBEDS: Have you ever used a sunlamp or gone to a tanning salon or solarium to get a suntan?

Yes ☐ (1) ⇒ a-d No ☐ (0) Don't know ☐ (9)

a) If YES, when did you first start using a sunlamp/sunbed _____

b) When was the last time you used a sunlamp/sunbed _____

c) And how often do you use it, on average? _____

d) Has your sunlamp/sunbed usage changed over time, and if so, how? _____

_____

UV TREATMENTS: Have you ever had special UV lamp treatment prescribed by your doctor for any medical condition, for example for psoriasis or for vitiligo?

Yes ☐ (1) ⇒ a) No ☐ (0) Don't know ☐ (9)

a) If YES:

How old were you when you first had this treatment? (*Column 3)*

How often did you receive the treatment? (*Column 5)*

How old were you when you stopped the treatment? (*Column 4)*

Did you have any further course of UV treatment after this first course? (*Complete additional rows)*

	CONDITION	Age started	Age stopped	Frequency of Rx
1				
2				
3				
4				
5				
6				

DIETARY QUESTIONNAIRE: the next series of questions related to the types of food you have eaten in the last year.

Special Diets: firstly, can I just check - are you on a special diet for any reason; I mean by that are there types
of food which you don't eat or types of food which you must eat?

Yes ☐ (1) ⇒ a) No ☐ (0) Don't know ☐ (9)

a) If YES:

Diabetic ☐ (1) Low salt ☐ (5)

No meat ☐ (2) No fish ☐ (6)

Low fat ☐ (3) High protein ☐ (7)

Gluten free ☐ (4) No dairy produce/low lactose ☐ (8)

Other (*specify*) _____

Changes: During the last year have there been any major changes in what you eat or in your food habits?

Yes ☐ (1) ⇒ a) No ☐ (0) ⇒ *Food questionnaire, overleaf*

a) If YES, what has changed?_____

_____

FOOD LIST: I am now going to ask you how often you eat various kinds of foods. We need to do this in quite a lot of detail, so that we can find out the variety of vitamins, proteins etc you have and the main sources of energy in your diet.

All the questions refer to your diet during the last year (so the last 12 months). I will give you lists of foods and for each food type I want you to tell me how often you have eaten it, on average. The choice of responses is on this card *(SHOW CARD)*. I will start with meat and meat products. Please estimate your food use as best you can.

(Food Tables; for shaded items, quantity per occasion is asked as well as frequency, and then the number per portions per week or per day is calculated)

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
MEAT **(medium serving)**	Never, <1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
Beef: roast, steak, mince, stew or casserole	1	2	3	4	5	6	7	8	9
Beefburgers	1	2	3	4	5	6	7	8	9
Pork: roast, chops, stew or slices	1	2	3	4	5	6	7	8	9
Lamb: roast, chops or stew	1	2	3	4	5	6	7	8	9
Chicken / other poultry (eg turkey)	1	2	3	4	5	6	7	8	9
Bacon	1	2	3	4	5	6	7	8	9
Ham	1	2	3	4	5	6	7	8	9
Corned beef, Spam, luncheon meats	1	2	3	4	5	6	7	8	9
Sausages	1	2	3	4	5	6	7	8	9
Savoury pies eg meat pies, pork pie, pasties, steak & kidney pie, sausage rolls	1	2	3	4	5	6	7	8	9
Liver pate, liver sausage	1	2	3	4	5	6	7	8	9
Any other meat product? If yes, specify and give frequency									
----------	1	2	3	4	5	6	7	8	9
----------	1	2	3	4	5	6	7	8	9
----------	1	2	3	4	5	6	7	8	9

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
FISH **(medium serving)**	Never, < 1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
Fried fish in batter, eg fish & chips	1	2	3	4	5	6	7	8	9
Fish fingers, fish cakes	1	2	3	4	5	6	7	8	9
Other white fish, fresh or frozen; eg cod, haddock, plaice, sole, halibut	1	2	3	4	5	6	7	8	9
Oily fish, fresh or canned; eg mackerel, kippers, tuna, salmon, sardines, herring	1	2	3	4	5	6	7	8	9
Shellfish; eg crab, prawns, mussels	1	2	3	4	5	6	7	8	9
Fish roe, taramasalata	1	2	3	4	5	6	7	8	9
Any other fish product? If yes, specify and give frequency									
----------	1	2	3	4	5	6	7	8	9
----------	1	2	3	4	5	6	7	8	9
----------	1	2	3	4	5	6	7	8	9

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
BREAD & SAVOURY BISCUITS **(one slice or biscuit)**	Never, < 1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
White bread and rolls	1	2	3	4	5	6	7	8	9
Brown bread and rolls	1	2	3	4	5	6	7	8	9
Wholemeal bread and rolls	1	2	3	4	5	6	7	8	9
Speciality bread; eg ciabatta, rye bread, pitta bread (specify)	1	2	3	4	5	6	7	8	9
Cream crackers, cheese biscuits	1	2	3	4	5	6	7	8	9
Crispbread eg Ryvita	1	2	3	4	5	6	7	8	9

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
CEREALS **(one bowl)**	Never, < 1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
Porridge, Readybrek S Y	1	2	3	4	5	6	7	8	9
Breakfast cereal eg cornflakes, muesli S Y	1	2	3	4	5	6	7	8	9

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
POTATOES, RICE, PASTA **(medium serving)**	Never, <1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
Potatoes - boiled, mashed, instant, jacket	1	2	3	4	5	6	7	8	9
Potatoes - chips	1	2	3	4	5	6	7	8	9
Potatoes - roast	1	2	3	4	5	6	7	8	9
Potato salad	1	2	3	4	5	6	7	8	9
White Rice	1	2	3	4	5	6	7	8	9
Brown rice	1	2	3	4	5	6	7	8	9
Lasagne, moussaka: made-up dish	1	2	3	4	5	6	7	8	9
White or green pasta - dried/fresh eg spaghetti, macaroni, noodles	1	2	3	4	5	6	7	8	9
Wholemeal pasta – dried/fresh	1	2	3	4	5	6	7	8	9
Tinned pasta	1	2	3	4	5	6	7	8	9
Pizza	1	2	3	4	5	6	7	8	9

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
DAIRY PRODUCTS	Never, <1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
Single or sour cream (tblspn)	1	2	3	4	5	6	7	8	9
Double or clotted cream (tblspn)	1	2	3	4	5	6	7	8	9
Low fat yoghurt, fromage frais (125g carton)	1	2	3	4	5	6	7	8	9
Full fat or Greek yoghurt (125g carton)	1	2	3	4	5	6	7	8	9
Dairy desserts (125g carton)	1	2	3	4	5	6	7	8	9
Cheese eg Cheddar, Brie, Edam (medium serving)	1	2	3	4	5	6	7	8	9
Cottage cheese, low fat soft cheese (medium serving)	1	2	3	4	5	6	7	8	9
Eggs: boiled/fried/scrambled etc (one)	1	2	3	4	5	6	7	8	9
Quiche (medium serving)	1	2	3	4	5	6	7	8	9
Low calorie, low fat salad cream (tblspn)	1	2	3	4	5	6	7	8	9
Salad cream, mayonnaise (tblspn)	1	2	3	4	5	6	7	8	9
French dressing (tblspn)	1	2	3	4	5	6	7	8	9
Other salad dressing (tblspn)	1	2	3	4	5	6	7	8	9

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
FAT ON BREAD OR VEGETABLES	Never, <1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
100% butter	1	2	3	4	5	6	7	8	9
Block margarine (hard)	1	2	3	4	5	6	7	8	9
Soft margarine (not low fat)	1	2	3	4	5	6	7	8	9
Low fat spread	1	2	3	4	5	6	7	8	9

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
SWEETS AND SNACKS **(medium serving)**	Never, <1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
Sweet biscuits, chocolate eg digestive (one)	1	2	3	4	5	6	7	8	9
Sweet biscuits, plain eg Nice, ginger (one)	1	2	3	4	5	6	7	8	9
Cakes eg fruit, sponge - home baked	1	2	3	4	5	6	7	8	9
Cakes eg fruit, sponge - ready made	1	2	3	4	5	6	7	8	9
Buns, pastries: eg scones, flapjacks - home-baked	1	2	3	4	5	6	7	8	9
Buns, pastries: eg croissants, doughnuts- ready-made	1	2	3	4	5	6	7	8	9
Fruit pies, tarts, crumbles: home-baked	1	2	3	4	5	6	7	8	9
Fruit pies, tarts, crumbles - ready-made	1	2	3	4	5	6	7	8	9
Sponge puddings - home-baked	1	2	3	4	5	6	7	8	9
Sponge puddings - packed mixes	1	2	3	4	5	6	7	8	9
Sponge puddings - ready-made	1	2	3	4	5	6	7	8	9
Rice pudding, semolina, tapioca	1	2	3	4	5	6	7	8	9
Blancmange, mousse, trifle, other milk puddings	1	2	3	4	5	6	7	8	9
Ice cream, choc ices	1	2	3	4	5	6	7	8	9
Chocolates, single or square	1	2	3	4	5	6	7	8	9
Chocolate snack bars eg Mars, Crunchie	1	2	3	4	5	6	7	8	9
Sweets, toffees, mints	1	2	3	4	5	6	7	8	9
Sugar added to tea, coffee, cereal (tspn)	1	2	3	4	5	6	7	8	9
Crisps or other packet snacks eg Wotsits	1	2	3	4	5	6	7	8	9
Peanuts or other nuts (50g)	1	2	3	4	5	6	7	8	9

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
SOUPS. SAUCES & SPREADS	Never, <1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
Vegetable soups (bowl) - home-made S Y	1	2	3	4	5	6	7	8	9
Vegetable soups (bowl) - packet S Y	1	2	3	4	5	6	7	8	9
Vegetable soups (bowl) - tinned S Y	1	2	3	4	5	6	7	8	9
Meat soups (bowl) - home-made S Y	1	2	3	4	5	6	7	8	9
Meat soups (bowl) - packet S Y	1	2	3	4	5	6	7	8	9
Meat soups (bowl) - tinned S Y	1	2	3	4	5	6	7	8	9
Sauces: eg white sauce, cheese sauce, gravy (tblspn)	1	2	3	4	5	6	7	8	9
Tomato ketchup (tablespoon)	1	2	3	4	5	6	7	8	9
Pickles, chutney (tablespoon)	1	2	3	4	5	6	7	8	9
Marmite, Bovril (tablespoon)	1	2	3	4	5	6	7	8	9
Jam, marmalade, honey (teaspn)	1	2	3	4	5	6	7	8	9
Peanut butter (teaspoon)	1	2	3	4	5	6	7	8	9

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
DRINKS	Never, <1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
Tea (cup)	1	2	3	4	5	6	7	8	9
Coffee, instant or ground (cup)	1	2	3	4	5	6	7	8	9
Coffee, decaffeinated (cup)	1	2	3	4	5	6	7	8	9
Coffee whitener eg Coffee-mate (teaspn)	1	2	3	4	5	6	7	8	9
Cocoa, hot chocolate (cup) S Y	1	2	3	4	5	6	7	8	9
Horlicks, Ovaltine, malt drink (cup) S Y	1	2	3	4	5	6	7	8	9
Wine (glass)	1	2	3	4	5	6	7	8	9
Beer, lager or cider (half pint)	1	2	3	4	5	6	7	8	9
Port, sherry, vermouth, liqueurs (glass)	1	2	3	4	5	6	7	8	9
Spirits eg gin, brandy, whisky, vodka (single)	1	2	3	4	5	6	7	8	9
Low calorie or diet fizzy drinks (glass)	1	2	3	4	5	6	7	8	9
Fizzy soft drinks; eg Coca Cola, lemonade	1	2	3	4	5	6	7	8	9
Pure (100%) fruit juice eg orange, apple juice	1	2	3	4	5	6	7	8	9
Fruit squash or cordial (glass)	1	2	3	4	5	6	7	8	9

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
FRUIT **(1 fruit or medium serving)**	Never, <1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
Apples, fresh	1	2	3	4	5	6	7	8	9
Oranges, satsumas, mandarins, clementines, fresh S Y	1	2	3	4	5	6	7	8	9
Grapefruit, fresh	1	2	3	4	5	6	7	8	9
Bananas, fresh	1	2	3	4	5	6	7	8	9
Grapes, fresh S Y	1	2	3	4	5	6	7	8	9
Melon, fresh S Y	1	2	3	4	5	6	7	8	9
Kiwi fruit, fresh	1	2	3	4	5	6	7	8	9
Pears, fresh S Y	1	2	3	4	5	6	7	8	9
Peaches, plums, apricots S Y	1	2	3	4	5	6	7	8	9
Strawberries/raspberries, fresh S Y	1	2	3	4	5	6	7	8	9
Any other fresh fruit? (specify)									
---------- S Y	1	2	3	4	5	6	7	8	9
---------- S Y	1	2	3	4	5	6	7	8	9
Tinned fruit	1	2	3	4	5	6	7	8	9
Dried fruit eg raisins, prunes, dates	1	2	3	4	5	6	7	8	9

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
VEGETABLES (Fresh or frozen) **medium serving**	Never, <1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
Carrots	1	2	3	4	5	6	7	8	9
Broccoli	1	2	3	4	5	6	7	8	9
Spring greens, kale	1	2	3	4	5	6	7	8	9
Cabbage	1	2	3	4	5	6	7	8	9
Peas	1	2	3	4	5	6	7	8	9
Cauliflower	1	2	3	4	5	6	7	8	9
Leeks	1	2	3	4	5	6	7	8	9
Onions	1	2	3	4	5	6	7	8	9
Garlic	1	2	3	4	5	6	7	8	9
Mushrooms	1	2	3	4	5	6	7	8	9
Sweet peppers	1	2	3	4	5	6	7	8	9
Beansprouts	1	2	3	4	5	6	7	8	9

CONTINUED.....

FOOD AND AMOUNTS	AVERAGE USE IN THE LAST YEAR								
VETETABLES (contd)	Never, <1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ x a day
Tomatoes	1	2	3	4	5	6	7	8	9
Beetroot	1	2	3	4	5	6	7	8	9
Coleslaw	1	2	3	4	5	6	7	8	9
Avocado	1	2	3	4	5	6	7	8	9
Spinach S Y	1	2	3	4	5	6	7	8	9
Brussels sprouts S Y	1	2	3	4	5	6	7	8	9
Green beans, broad beans, runner beans S Y	1	2	3	4	5	6	7	8	9
Marrow, courgettes S Y	1	2	3	4	5	6	7	8	9
Parsnips, turnips, swedes S Y	1	2	3	4	5	6	7	8	9
Green salad, lettuce, cucumber celery S Y	1	2	3	4	5	6	7	8	9
Watercress S Y	1	2	3	4	5	6	7	8	9
Sweetcorn S Y	1	2	3	4	5	6	7	8	9
Any other vegetables? (specify)									
---------- S Y	1	2	3	4	5	6	7	8	9
---------- S Y	1	2	3	4	5	6	7	8	9
Tinned vegetables	1	2	3	4	5	6	7	8	9
Baked beans	1	2	3	4	5	6	7	8	9
Dried lentils, beans, peas	1	2	3	4	5	6	7	8	9
Tofu, soya meat, TVP, vegeburger	1	2	3	4	5	6	7	8	9

Are there any OTHER foods which you ate regularly? Yes ☐ No ☐

If YES, list below:

Food	**Usual Serving Size**	**Frequency eaten**
----------	----------	----------
----------	----------	----------
----------	----------	----------
----------	----------	----------
----------	----------	----------

What type of milk do you most often use (select one)?

Full cream	☐ (1)	Soya	☐ (5)
Semi-skimmed	☐ (2)	Dried milk	☐ (6)
Skimmed	☐ (3)	Other _____	☐ (7)
Channel Islands	☐ (4)	None	☐ (0)

How much milk do you drink each day, including milk with tea, coffee, cereals etc:

None	☐ (0)	Three quarters of a pint	☐ (3)
Quarter of pint	☐ (1)	One pint	☐ (4)
Half a pint	☐ (2)	More than one pint	☐ (5)

Did you usually eat breakfast cereal (other than porridge and Ready Brek mentioned earlier)?

Yes ☐ (1) No ☐ (0)

If YES, which brand and type of breakfast cereal, including muesli did you usual eat?
List the one or two types most often used

Brand	**Type**
---	---
---	---

What kind of fat, if any, did you most often use for frying, roasting, grilling etc (*Select one only)*?

None	☐ (0)	Vegetable oil	☐ (3)
Butter	☐ (1)	Solid vegetable fat	☐ (4)
Lard / dripping	☐ (2)	Margarine	☐ (5)

If you used vegetable oil, please give type (eg corn, sunflower)_____

What kind of fat, if any, did you most often use for baking cakes etc?

None	☐ (0)	Vegetable oil	☐ (3)
Butter	☐ (1)	Solid vegetable fat	☐ (4)
Lard / dripping	☐ (2)	Margarine	☐ (5)

What kind of fat, if any, did you most often use for spreading on bread or adding to vegetables? *Specify precise brand*, eg Anchor Half Fat Spread, Delight Extra Low, Flora, Flora Extra Light etc

---

Have you taken any vitamins, minerals, fish oils, fibre or other food supplements during the past year?

Yes ☐ (1) No ☐ (0) Don't know ☐ (9)

If YES, complete the table below. If more than five types of supplement have been taken, put the most frequently consumed brands first

Vitamin Supplements	Average frequency **Tick one box per line to show how often on average supplements are consumed**									
Name and brand *List full name, brand and strength*	Dose: no of pills capsules,teaspns consumed	Never, <1x a month	1-3x a month	Once a week	2-4x a week	5-6x a week	Once a day	2-3x a day	4-5x a day	6+ a day

Smoking: Can I now ask about smoking - which best describes your smoking habits (including cigar or pipe smoking?):

Current smoker (either regular or occasional) ☐ (1)

Never at any time been a regular smoker ☐ (0)

Ex-smoker: smoked regularly in the past ☐ (2) Gave up _____ yrs ago

Have you smoked any cigarettes at all in the last 12 months?

Yes, one or more ☐ (1) ⇒ f), below

No, none ☐ (0)

f) If Yes, number of cigarettes smoked on average over the last year:

Can be given as either Cigarettes per day _____ or
Cigarettes per week _____ or
Total cigarettes during the year _____ or

Stress: can I now ask about any stressful events that you may have experienced in the last year?

- Firstly can I ask about whether anyone close to you has died in the last year?

A husband/wife or partner? Yes ☐ (1) ⇒ when was this?_____months ago No ☐ (0)

Another close family member ? Yes ☐ (1) ⇒ _____months ago No ☐ (0)

A close friend? Yes ☐ (1) ⇒ _____months ago No ☐ (0)

- Can I ask about whether any one close to you have been seriously ill?

A husband/wife or partner? ? Yes ☐ (1) ⇒ when was this?_____months ago No ☐ (0)

Another close family member ? Yes ☐ (1) ⇒ _____months ago No ☐ (0)

A close friend? Yes ☐ (1) ⇒ _____months ago No ☐ (0)

- Or any other other events you found stressful?

Divorce / separation Yes ☐ (1) ⇒ when was this?_____months ago No ☐ (0)

Difficulties with other family members Yes ☐ (1) ⇒ _____months ago No ☐ (0)

Difficulties with neighbours Yes ☐ (1) ⇒ _____months ago No ☐ (0)

Serious financial worries Yes ☐ (1) ⇒ _____months ago No ☐ (0)

Moving house Yes ☐ (1) ⇒ _____months ago No ☐ (0)

Problems at work Yes ☐ (1) ⇒ _____months ago No ☐ (0)

- Are there any other events which stand out as having caused you stress in the last year?

Yes ☐ (1) (specify) _____ _____months ago No ☐ (0)

Trauma: have you had any physical injuries in the last six months, for example any falls, or knocks which resulted in bruising, or any other injuries?

Yes ☐ (1) ⇒ (a) No ☐ (0) Don't know ☐ (9)

a) If Yes, how long ago was this, and what sort of injury was it? *(Table, below)*

	When?	Site of Injury	Details
1			
2			
3			
4			

Finally, if I could ask a couple of general questions:

Housing Tenure: regarding your current accommodation, do you (and/or your spouse/partner):

Own your present home ☐ (1)

Rent your home from the local council ☐ (2)

Rent your home from a housing association ☐ (3)

Rent your home privately or are rent free ☐ (4)

Other ☐ (5) *Specify*_____

Car access: is there a car or van available for use by you or other members of your household?

Yes, one ☐ (1)

Yes, two or more ☐ (2)

No, none ☐ (0)

END OF INTERVIEW - *Thank subject for help*

INTERVIEW DETAILS

Length of interview: ☐☐ minutes

Interview completed: Yes ☐(1) No ☐(0) ⇒(a)

a) If interview curtailed, please give reasons _____

_____

Did anyone help with the interview?

Yes (proxy for subject) ☐(1) ⇒(b) Yes (subject completed, with help) ☐(2) ⇒(b) No ☐(0)

b) If 'Yes', relationship to subject of others helping with interview:

Lives with subject: Yes ☐(1) No ☐(0)

Relationship: Spouse ☐(1) Other relative ☐(2) Other (please specify)_____ (3)

SUBJECT'S MEMORY OF PAST HISTORY:

	Poor	Fair (some problems)	Good	Declined to answer	Comments
Sun exposure					
Diet					
Personal/work history					
Contacts with children / chickenpox					

Anthropometry:

Weight _____ Kg Height _____ cm Demispan _____ cm

_____ cm

Sample taken?

Yes ☐(1) ⇒ Specimen No. _____ No ☐(0) ⇒ Reason?_____

RESIDENCE CALENDAR

Start by writing in the left-hand column the name of the village/town and country where you were born, and in the right-hand column the dates (period of time) you lived there

Start a new row for each time you moved to a different area - please include in the list any places you visited for more than six months

You do not need to include moves within the same town or city

If you lived in a village or a small town, please write down the county as well, to help us identify where the village was.

If you lived outside the UK, please write down the name of the country as well.

Please see the example of a completed residence calendar overleaf *(provided in original letter)*

TOWN (& country if outside the UK)	PERIOD COVERED month/year - month/year

JOB CALENDAR

Start with the time when you were 14 years old. In the left-hand column of the first row, write down your job title or (if appropriate) school pupil. In the middle column, write down the dates (the period of time) that you were in that job. In the right-hand column, write down how many days a week you did this job (for example, 5 days/week).

Start a new row for each time your job or main activity changed - for example, if you worked for the same company for 20 years, first working in the office and then working as a sales rep, this would count as two different jobs (two different lines in the job calendar)

Please include voluntary work, times when you were at college or university, and times when you were not employed or were looking after the home and children.

Please leave out any jobs you did for less than 6 months.

Please see the example of a completed job calendar overleaf *(provided in original letter)*

JOB (including student, house-wife, unemployed, retired)	PERIOD COVERED month/year - month/year	Number of days worked per week

UV EXPOSURE IN LAST 20 YEARS

Working days Job title Warmer (hrs/day) Cooler (hrs/day)	20 yrs ago	10 yrs ago	Last year	(Other)	(Other)	(Other)
Non-working days Warmer (hrs/day) Cooler (hrs/day)						
Holidays - Apr/Sept How often? (*code*) Which country? How many days? Exposure 9-5			(include dates)	N/A	N/A	N/A
Holidays - Oct/Mar How often? (*code*) Which country? How many days? Exposure 9-5			(include dates)	N/A	N/A	N/A
Job/non-work days Freq hat Freq prot clothes Holidays Freq hat Freq prot clothes						

@Contacts with varicella or with children and protection against herpes zoster in adults: a case-control study

Sara L Thomas, Jeremy G Wheeler, Andrew J Hall

Summary

Background Whether exogenous exposure to varicella-zoster-virus protects individuals with latent varicella-zoster virus infection against herpes zoster by boosting immunity is not known. To test the hypothesis that contacts with children increase exposure to varicella-zoster virus and protect latently infected adults against zoster, we did a case-control study in south London, UK.

Methods From 22 general practices, we identified patients with recently diagnosed zoster, and control individuals with no history of zoster, matched to patients by age, sex, and practice. Participants were asked about contacts with people with varicella or zoster in the past 10 years, and social and occupational contacts with children as proxies for varicella contacts. Odds ratios were estimated with conditional logistic regression.

Findings Data from 244 patients and 485 controls were analysed. On multivariable analysis, protection associated with contacts with a few children in the household or via childcare seemed to be largely mediated by increased access to children outside the household. Social contacts with many children outside the household and occupational contacts with ill children were associated with graded protection against zoster, with less than a fifth the risk in the most heavily exposed groups compared with the least exposed. The strength of protection diminished after controlling for known varicella contacts; the latter remained significantly protective (odds ratio 0·29 [95% CI 0·10–0·84] for those with five contacts or more).

Interpretation Re-exposure to varicella-zoster virus via contact with children seems to protect latently infected individuals against zoster. Reduction of childhood varicella by vaccination might lead to increased incidence of adult zoster. Vaccination of the elderly (if effective) should be considered in countries with childhood varicella vaccination programmes.

Lancet 2002; **360:** 678–82. Published online July 2, 2002
http://image.thelancet.com/extras/01art6088web.pdf

Infectious Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK
(S L Thomas MSc, J G Wheeler MSc, A J Hall FRCP)

Correspondence to: Dr Sara L Thomas
(e-mail: Sara.Thomas@lshtm.ac.uk)

Introduction

Primary infection with varicella-zoster virus causes varicella, after which the virus establishes latency in dorsal root ganglia.[1–3] Reactivation of latent infection is thought to result from declining specific cell-mediated immunity, and leads to herpes zoster.[4–6] Zoster occurs frequently in ageing populations and causes substantial acute and chronic morbidity, the commonest long-term complication being persistent pain (post-herpetic neuralgia).[7]

Hope-Simpson postulated that exogenous exposure to people with varicella or zoster might boost specific immunity and therefore decrease the risk of zoster in latently infected individuals.[8] Mothers of children with varicella have cell-mediated immune boosting, and children with leukaemia seem to be protected against zoster by household exposure to varicella.[9,10] However, whether exogenous exposure protects against zoster in immunocompetent adults is unclear. In one study, paediatricians had more contacts with patients infected with varicella-zoster virus than dermatologists or psychiatrists, and were significantly less likely to have developed zoster, but the results could have been influenced by very low response rates to the survey.[11]

The role of immune boosting is an important issue for varicella vaccination programmes, since a reduction in childhood varicella will result in fewer exogenous exposures to varicella-zoster virus, which could lead to increased incidence of zoster among unvaccinated adults.[12] Varicella vaccination has already been introduced in countries such as the USA and Japan, and is being considered by many European countries. We therefore set up a study to test the hypothesis that exogenous exposure to varicella-zoster virus protects against zoster.

Methods

Patients and controls

This investigation was one objective of a community-based case-control study of risk factors for zoster in immunocompetent adults in south London, UK, between September, 1997, and December, 1998. A reporting system was set up among 22 general practices to identify individuals who had recently been diagnosed with zoster by their family physician. For each patient with zoster, two controls with no history of zoster were sought by searching practice registers for individuals who were nearest in age to the patient, and matched for sex and practice. Patients and potential controls were approached and invited to take part in the study. Those who agreed and were eligible were interviewed at home. Ethics approval was obtained from the London School of Hygiene and Tropical Medicine Ethics Committee, and from four local research ethics committees. All participants gave written informed consent.

Cases of zoster were confirmed where possible by use of PCR to detect varicella-zoster virus DNA in vesicular fluid or crust samples obtained at interview.[13] Unconfirmed cases were divided into "probable" and "other" groups with standardised diagnostic criteria applied at interview. Probable cases had a unilateral vesicular or maculopapular rash with a dermatomal distribution where either rash or pain covered at least half the dermatome, or rash and pain were less extensive, but pain lasted at least 1 month after rash onset. Patients with a history of a similar dermatomal rash at any site within the past 10 years were excluded from the probable group. In this study, only data from confirmed and probable cases and their matched controls were analysed.

Patients and controls were excluded if they were younger than 16 years; had a cell-mediated immunosuppressive disorder or therapy in the past 6 months or a history of active cancer in the past 5 years; were of African ethnic origin (a group at higher risk of undiagnosed HIV infection in this population);[14] were temporarily registered with the practice; or were incapable of answering questions. Patients were also excluded if they were identified more than 8 weeks after rash onset. Controls were excluded if they had a history of zoster.

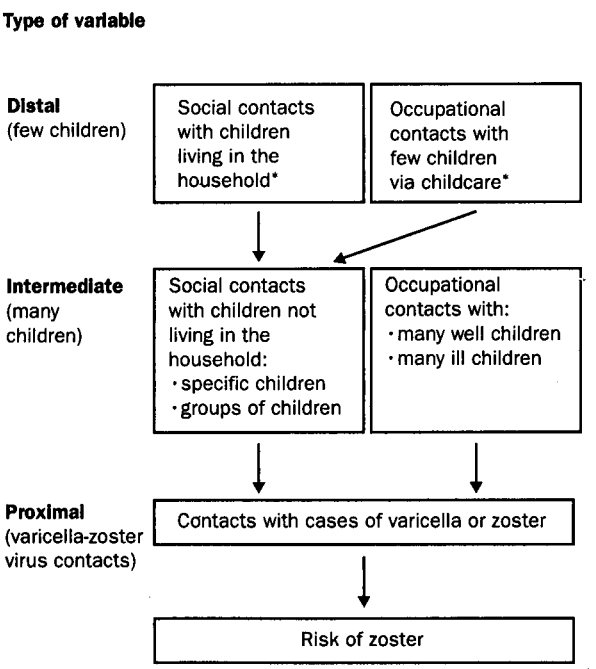
Data collection

Participants were asked about contacts with people with varicella or zoster in the past 10 years. Because varicella is mostly acquired before the age of 10 years in the UK,[15] additional data were sought on contacts with children aged 1–10 years as surrogates for exogenous varicella exposures. We asked questions about social contacts with children in the past 10 years, including (1) specific children living in the household, (2) specific children not living in the household (such as grandchildren and neighbours), or (3) a range of different children in groups with changing membership (such as at school playgrounds or parties). For each child, we sought information on the average frequency (per week or per month) and duration (in years) of contact. We also asked about the duration of occupational exposure to children, either with (4) a few specific children through childcare (ĕg, through childminding, full-time parenting), (5) with many well children (eg, through teaching), or (6) with many ill children (eg, through being a doctor). These data were used to create three types of exposure: exposure to people with varicella or zoster, exposure to a few children (1 and 4, above), or exposure to many children (2, 3, 5, and 6). Information on potential confounders included ethnic origin, lifetime country of residence, and socioeconomic factors (household tenure and car ownership).

We calculated the total number of social contacts with children in the past 10 years by multiplying the average frequency of contact by duration of contact for each child, and summing the results. Social contacts were then grouped into "none" and into two, three, or five "exposed" groups, each of which contained an equal number of controls (quantiles of exposure). Duration of occupational exposure was divided into "none", "up to 5 years", and "more than 5 years" of exposure.

Statistical analysis

Sample-size calculations for the entire study were derived from standard equations for matched case-control studies, adjusted for our choice of two controls per case.[16] Taking an odds ratio of 2·0, a minimum 10% prevalence of exposure in controls, 90% power at 5% significance (two-sided), and a 20% increase to accommodate multivariable analyses, we needed to assess 244 confirmed and probable cases, and 488 controls.



Conceptual framework for modelling effect of contacts with children or with patients with varicella or zoster on risk of zoster

*Parents who stayed at home to look after children full-time appear in both distal groups.

We set out to test the hypothesis that contacts with children protected latently infected adults against zoster, and that this protection resulted from increased exposure to varicella-zoster virus. Analyses were done with Stata statistical software, version 6.0 (StataCorp, College Station, TX, USA). Odds ratios were estimated by conditional logistic regression, with zoster as the outcome variable. The significance of associations between exposure variables and risk of zoster was calculated with likelihood ratio tests of heterogeneity and of linear trend; 95% CIs were calculated with Wald-based SEs. Univariable analyses identified variables associated with zoster to the significance level of $p \leq 0{\cdot}2$ for initial inclusion in multivariable models.

Variables were classified as distal, intermediate, or proximal, according to their position in the proposed chain of causation as outlined in the figure.[17] In this conceptual framework, varicella or zoster exposures had a direct effect on the risk of zoster, and were categorised as proximal variables. Social or occupational contacts with many children that were likely to result in varicella exposures were categorised as intermediate variables. Contacts with a few children living in the household or via childcare work were categorised as distal variables because some of their effect might be mediated through contacts with a wider range of children outside the household (the intermediate variables). Distal variables were added first to the multivariable model, and retained as long as they remained significantly associated with zoster ($p \leq 0{\cdot}1$). Intermediate variables

Distal variables	Patients (n=244)	Controls (n=485)	Univariable odds ratio (95% CI)	p	Adjusted for intermediate social child contacts	p
Childcare work with a few specific children						
None	233 (95·5%)	436 (89·9%)	1·00		1·00	
≤5 years' duration	10 (4·1%)	28 (5·8%)	0·37 (0·13–1·06)		0·94 (0·27–2·99)	
>5 years' duration	1 (0·4%)	21 (4·3%)	0·06 (0·01–0·50)	0·0004	0·19 (0·02–1·79)	0·214 0·169 (trend)
Number of child-day contacts with children living in household						
None	202 (82·8%)	355 (73·2%)	1·00		1·00	
7–2550*	27 (11·1%)	65 (13·4%)	0·62 (0·37–1·05)		0·96 (0·54–1·69)	
2551–14 901	15 (6·1%)	65 (13·4%)	0·34 (0·18–0·64)	0·001	0·71 (0·34–1·47)	0·638 0·403 (trend)

*Quantiles of exposure, see methods.

Table 1: **Effects on risk of zoster of contacts with limited numbers of children living in household and via childcare work in past 10 years**

were added second, to demonstrate the extent to which they explained the effect of distal variables, then proximal variables were added to determine whether they explained distal and intermediate factors. Variables excluded at the univariable or distal stages of analysis were added again at the proximal stage to assess whether they became significantly associated with zoster in the presence of other variables. Confounding variables were added to the model if they changed any of the effect estimates of interest by 10% or more.[18] Interactions between contact variables and age were investigated in the final model.

Role of the funding source

Neither funder of this study was involved in the study design; the collection, analysis, and interpretation of data; the writing of the report; or the decision to submit the paper for publication.

Results

During the study period, 436 patients were identified, of whom 139 were ineligible (46 were younger than 16 years, 37 had recent immunosuppression, 18 were African, 11 were temporarily registered, four were incapable of answering questions, and 23 were identified more than 8 weeks after rash onset). Of the remaining 297 patients, 16 (5·4%) were not enrolled: 12 refused and four were away from London or repeatedly unavailable for more than 8 weeks. The eligibility of these patients was not ascertained. The remaining 281 patients were categorised as confirmed (92), probable (152), or other (37) cases. Of the confirmed and probable cases, 107 (43·9%) were men, and the median age was 57·2 years (range 16·5–91·2).

488 controls were needed for the 244 confirmed and probable cases. Letters were sent to 895 individuals, of whom 162 were ineligible (118 had a history of zoster, 22 had recent immunosuppression, 11 were African, one was temporarily registered, and 10 were incapable of answering questions). A further 145 were unsuitable (106 no longer lived in London, 22 were living away for extended periods, 11 were dead, and six had an incorrect date of birth on practice records). Of the remaining 588 potentially eligible individuals, 103 (17·5%) were not included in the study (75 refused, nine twice cancelled interviews, three were in hospital, one had a non-existent address, and 15 could not be contacted after more than four attempts). The remaining 485 controls were enrolled; for three of the enrolled patients only one matched control was obtained. The mean difference in age between patients and their matched controls was 4·7 days. Controls were interviewed a median of 35 days after patients.

Contact with a few children living in the household or via childcare work in the past 10 years was strongly associated with protection against zoster on univariable analysis, with evidence of a dose-response effect. However, neither distal variable remained significantly associated with risk of zoster after adjusting for the effects of social contacts with specific children not living in the household and children in groups (table 1). Childcare and household contact variables were therefore dropped from the model.

Table 2 lists univariable effect estimates for the intermediate child contact variables, in the 10 years before interview, that were associated with zoster. Protection increased with longer duration of occupational exposure to many ill children, and with greater numbers of social contacts with specific children not living in the household or children in groups. There was no significant association between duration of occupational exposure to many well children in the past 10 years and risk of zoster, even after analyses were restricted to individuals working in primary-school or nursery settings (odds ratio 0·94 [95% CI 0·47–1·87]). Intermediate child contact variables remained significantly associated with protection against zoster after adjusting for each other and for ethnic origin, with little change to the effect estimates (data not shown). However, the strength of associations between intermediate child contacts and zoster decreased after adjusting for contact with known cases of varicella (table 2), remaining most strongly significant for contacts with children in groups.

Contact with people with varicella in the past 10 years was strongly associated with protection against zoster on univariable analysis (table 2), and this association remained after adjusting for occupational and social child contacts. Contact with people with zoster was weakly associated with protection against zoster on univariable analysis, but not significantly associated with zoster in the final model (table 2). Ethnic origin slightly confounded the effect of occupational exposure to ill children, and was added to all models. After adding ethnic origin, childhood residence in the tropics and socioeconomic variables made little difference to effect estimates for the variables of interest. The effect of the contact variables did not vary with participants' age (p for interaction >0·3 for all).

We could not test study participants for HIV infection, and so could not confirm that all cases and controls were HIV-negative. The effect of child contacts might be confounded by undiagnosed HIV infection in patients. Homosexual men in London are a group at high risk of HIV infection (which increases their chance of developing zoster), and could have relatively few child

	Patients (n=244)	Controls (n=485)	Univariable odds ratio (95% CI)	p	Adjusted for other intermediate variables and varicella contacts*	p
Intermediate variables						
Number of social contacts with specific children not living in household						
None	30 (12·3%)	49 (10·1%)	1·00		1·00	
2–107†	60 (24·6%)	87 (17·9%)	1·02 (0·59–1·81)		1·03 (0·57–1·85)	
108–420	53 (21·7%)	88 (18·1%)	0·91 (0·51–1·62)		0·94 (0·52–1·73)	
421–1334	52 (21·3%)	87 (18·9%)	0·89 (0·49–1·63)		0·90 (0·48–1·70)	
1335–3457	30 (12·3%)	87 (18·9%)	0·53 (0·28–0·98)		0·60 (0·30–1·17)	
3458–32 631	19 (7·8%)	87 (17·9%)	0·30 (0·14–0·63)	0·0003	0·43 (0·19–0·94)	0·079
						0·007 (trend)
Number of social contacts with children in groups						
None	197 (80·7%)	308 (63·5%)	1·00		1·00	
6–550†	24 (9·8%)	59 (12·2%)	0·63 (0·38–1·06)		0·72 (0·41–1·27)	
551–3652	16 (6·6%)	59 (12·1%)	0·32 (0·17–0·62)		0·44 (0·22–0·89)	
3653–45 023	7 (2·9%)	59 (12·2%)	0·12 (0·06–0·35)	<0·0001	0·19 (0·07–0·50)	0·001
						0·0001 (trend)
Occupational contact with many ill children						
None	241 (98·8%)	460 (94·8%)	1·00		1·00	
≤5 years' duration	2 (0·8%)	14 (2·9%)	0·26 (0·06–1·17)		0·25 (0·05–1·20)	
>5 years' duration	1 (0·4%)	11 (2·3%)	0·17 (0·02–1·29)	0·015	0·27 (0·03–2·51)	0·062
						0·025 (trend)
Proximal variables						
Number of known varicella contacts						
None	179 (73·4%)	283 (58·4%)	1·00		1·00‡	
1	34 (13·9%)	74 (15·3%)	0·67 (0·42–1·08)		0·90 (0·54–1·52) ‡	
2	20 (8·2%)	45 (9·3%)	0·61 (0·34–1·09)		0·83 (0·45–1·56) ‡	
3–4	6 (2·5%)	44 (9·1%)	0·15 (0·06–0·39)		0·26 (0·10–0·72) ‡	
≥5	5 (2·0%)	39 (8·0%)	0·14 (0·05–0·39)	0·0001	0·29 (0·10–0·84) ‡	0·016
						0·003 (trend)
Number of known zoster contacts						
None	189 (77·5%)	338 (69·7%)	1·00		1·00§	
1	44 (18·0%)	110 (22·7%)	0·71 (0·48–1·05)		0·79 (0·51–1·23)§	
≥2	11 (4·5%)	37 (7·6%)	0·51 (0·25–1·04)	0·052	0·92 (0·42–2·03)§	0·581

*Also adjusted for ethnic origin. †Quantiles of exposure, see methods. ‡Adjusted for intermediate variables and ethnic origin. §Adjusted for intermediate variables, varicella contacts, and ethnic origin.

Table 2: **Effects on risk of zoster of contacts with many children and exgenous exposure to varicella-zoster virus in the past 10 years**

contacts.[14,19] Multivariable analyses were therefore repeated in two subgroups of individuals at low risk of HIV infection: first women and then all individuals older than 60 years. Statistical power was reduced, but protective trends associated with social and occupational child contacts were similar to those shown in the whole dataset (data available on request). The effect of imperfect specificity of the probable zoster case definition was also investigated by repeating analyses in the subset of confirmed cases and their matched controls. Similar protective patterns were shown.

Discussion

The findings from this study suggest that continued exogenous exposure to varicella is protective against zoster in latently infected adults. This result is consistent with those of Gershon and colleagues,[10] who found that vaccinated children with leukaemia were at significantly lower risk of zoster if they had household exposure to varicella, and that many of these children had evidence of immunological boosting. In our study, there were dose-response effects associated with a range of occupational and social exposures to children and with varicella contacts. Results of analyses using a hierarchical model-building strategy (figure) show that living with children seems to protect against zoster largely by increasing access to a range of other children outside the household, and that the protection afforded by contacts with many children seems to be largely explained by exposure to varicella-zoster virus. The latter conclusion is supported by analyses showing that protection against zoster is strongest when contacts are with children in groups of changing membership in occupational or social settings (increasing the likelihood of contacting a case of varicella).

Some protective effect of child contacts remained after adjustment for known varicella contacts. This effect might represent unrecognised or forgotten contacts with children with varicella, since varicella is infectious before rash onset, and this is especially likely for social contacts with children in groups of changing membership.[20] If this explanation is correct, the total protective effect of (known and unknown) varicella contacts will be greater than that estimated in the final model, which represents only the effect of known varicella contacts independent of the effect of unknown contacts. Interestingly, occupational contact with many well children (eg, through teaching) was not protective against zoster. Perhaps varicella contacts are more distant in these settings than in social settings, and are more limited in duration if children with varicella are absent from school while experiencing rash. Contact with zoster cases was not associated with protection against zoster. This finding is less surprising, since zoster is less infectious than varicella and most zoster contacts had rash on non-exposed areas of the body.

Other explanations for the protective effect of child contacts should be considered. First, ethnic origin is a potential confounder of the effect of child contacts on risk of zoster, since some ethnic groups could be at lower risk of zoster than others and have greater contacts with children via extended families.[21] However, neither ethnic origin nor country of residence in childhood accounted for the protective effect of child contacts in this study.

Second, subgroup analyses indicated that the protective effect of child contacts was unlikely to result from undetermined HIV infection or misdiagnosis of zoster cases. Incomplete reporting of cases by practices might introduce bias if general practitioners were more likely to report cases with fewer child contacts. This situation is unlikely, since the overall study was investigating various risk factors for zoster, and investigation of a limited number of unreported cases showed that failure to be reported was due to general under-reporting by some practices or by specific practitioners, rather than selective reporting of cases. Participation by controls was high (82·5%), but some bias might have been introduced if those who refused or could not be contacted were eligible for inclusion and had fewer contacts with children.

Recall bias occurs in case-control studies if patients remember past exposures differently from controls. In this study, recall bias might have led to underestimation of the protective effects of child and varicella contacts. Many patients believed that zoster resulted from contact with cases of varicella or zoster, and had spent time trying to remember any contacts that might have infected them. Another concern in case-control studies is that having the disease affects exposure—in this situation, contact with children. However, reverse causality is unlikely to explain the findings. First, most patients were interviewed within 2 weeks of rash onset. Second, the number of child contacts was calculated from the average frequency before onset of rash, not the frequency in the past few days. For example, a patient who saw her grandchild on average once a week in the past year would be assigned 52 child contacts, even if she had not seen the child since onset of rash.

Children who are vaccinated against varicella might be at lower risk of later developing zoster.[22] Therefore, widespread varicella vaccination programmes might eventually decrease the incidence of zoster. However, the results of this study suggest that vaccination of children against varicella could lead to a prolonged period of increased incidence of zoster among unvaccinated adults, as a result of fewer exogenous exposures to varicella-zoster virus. This increase in incidence might have started already in countries such as the USA, but could be unrecognised due to limited surveillance of zoster. In view of this possibility, we should consider whether childhood varicella vaccination programmes should be expanded to include vaccination of older adults, to protect them against zoster. The results of the current US multicentre trial of varicella vaccination in elderly individuals will indicate whether this is a feasible approach.[23]

Contributors

Andrew Hall conceived and co-designed the study, and participated in the statistical analyses, interpretation of findings, and writing of the paper. Sara Thomas co-designed and ran the study, did the interviews, managed the data, designed and carried out the statistical analyses, interpreted the findings, and wrote the paper. Jeremy Wheeler participated in the design of the study, the statistical analyses and interpretation of the findings, and the writing of the paper.

Conflict of interest statement

Andrew Hall has received a contribution towards research funding from Merck, Sharp and Dohme (a vaccine manufacturer).

Acknowledgments

We thank all the individuals who agreed to take part in the study, and the participating general practices: Albion Street Health Centre; Aylesbury Partnership (all three sites); Belmont Hill Surgery; Brixton Hill Group Practice; Camberwell Green Surgery; Crown Dale Medical Centre; Grange Road Practice; Downham Way Surgery; Forest Hill Group Practice; Isidore Crown Health Centre; Jenner Health Centre; Lee Road Surgery; Mawbey Brough Health Centre; New Surgery, Clapham; Paxton Green Health Centre; Queens Road Partnership (both sites); Sternhall Lane Surgery; Sydenham Green Group Practice; and Torridon Road Surgery. We also thank Judy Breuer for helpful discussions on the diagnosis of zoster, and for doing the PCR analyses. Sara Thomas was funded initially by a research studentship from the Medical Research Council (UK), and then by the Research Foundation for Microbial Diseases, Osaka University, Japan.

References

1 Gilden DH, Vafai A, Shtram Y, et al. Varicella-zoster virus DNA in human sensory ganglia. *Nature* 1983; **306:** 478–80.
2 Furuta Y, Takasu T, Fukuda S, et al. Detection of varicella-zoster virus DNA in human geniculate ganglia by polymerase chain reaction. *J Infect Dis* 1992; **166:** 1157–59.
3 Kennedy PGE, Grinfeld E, Gow JW. Latent varicella-zoster virus is located predominantly in neurons in human trigeminal ganglia. *Proc Natl Acad Sci USA* 1998; **95:** 4658–62.
4 Arvin AM. Cell-mediated immunity to varicella-zoster virus. *J Infect Dis* 1992; **166** (suppl 1): S35–41.
5 Burke IL, Steele RW, Beard OW, et al. Immune responses to varicella-zoster in the aged. *Arch Intern Med* 1982; **142:** 291–93.
6 Berger R, Florent G, Just M. Decrease of the lymphoproliferative response to varicella-zoster virus antigen in the aged. *Infect Immun* 1981; **32:** 24–27.
7 Gilden DH, Kleinschmidt-DeMasters BK, LaGuardia JJ, Mahalingam R, Cohrs RJ. Medical progress: neurologic complications of the reactivation of varicella-zoster virus. *N Engl J Med* 2000; **342:** 635–45.
8 Hope-Simpson RE. The nature of herpes zoster: a long-term study and a new hypothesis. *Proc R Soc Med* 1964; **58:** 9–20.
9 Arvin AM, Koropchak CM, Wittek AE. Immunological evidence of re-infection with varicella zoster virus. *J Infect Dis* 1983; **148:** 200–05.
10 Gershon AA, LaRussa P, Steinberg S, et al. The protective effect of immunologic boosting against zoster: an analysis in leukemic children who were vaccinated against chickenpox. *J Infect Dis* 1996; **173:** 450–53.
11 Solomon BA, Kaporis AG, Glass AT, Simon SI, Baldwin HE. Lasting immunity to varicella in doctors (L.I.V.I.D study). *J Am Acad Derm* 1998; **38:** 763–65.
12 Garnett GP, Grenfell BT. The epidemiology of varicella-zoster virus infections: the influence of varicella on the prevalence of herpes zoster. *Epidemiol Infect* 1992; **108:** 513–28.
13 Nahass GT, Goldstein BA, Zhu WY, et al. Comparison of Tzanck smear, viral culture and DNA diagnostic methods in detection of herpes simplex and varicella-zoster infection. *JAMA* 1992; **18:** 2541–44.
14 Unlinked Anonymous Surveys Steering Group. Prevalence of HIV and hepatitis infections in the United Kingdom 1999. London: Department of Health, Public Health Laboratory Service, Institute of Child Health (London), Scottish Centre for Infection and Environmental Health, 2000.
15 Fairley CK, Miller E. Varicella-zoster virus epidemiology—a changing scene? *J Infect Dis* 1996; 174 (suppl 3): S314–19.
16 Schlesselman JJ. Case-control studies: design, conduct, analysis. New York: Oxford University Press, 1982.
17 Victora CG, Huttley SR, Fuchs SC, Olinto MT. The role of conceptual frameworks in epidemiological analysis: a hierarchical approach. *Int J Epidemiol* 1997; **26:** 224–27.
18 Maldonado G, Greenland S. Simulation study of confounder-selection strategies. *Am J Epidemiol* 1993; **138:** 923–36.
19 Buchbinder SP, Katz MH, Hessol NA, et al. Herpes zoster and human immunodeficiency virus infection. *J Infect Dis* 1992; **166:** 1153–56.
20 Weller TH. Varicella-herpes zoster virus. In: Evans AS, Kaslow RA, eds. Viral infections of humans: epidemiology and control, 4th edn. New York: Plenum Medical Book Co, 1997: 865–92.
21 Schmader K, George LK, Burchett BM, Hamilton JD, Pieper CF. Race and stress in the incidence of herpes zoster in older adults. *J Am Geriatr Soc* 1998; **46:** 973–77.
22 Kraus PR, Klinman DM. Efficacy, immunogenicity, safety, and use of live attenuated chickenpox vaccine. *J Pediatr* 1995; **127:** 518–25.
23 Oxman MN. Immunization to reduce the frequency and severity of herpes zoster and its complications. *Neurology* 1995; **45** (suppl 8): S41–46.